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No. 1

## LIPID METABOLISM AND DEVELOPMENT OF ANEMIA IN SPLENECTOMIZED GUINEA PIGS FED CHOLESTEROL<sup>1,2</sup>

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Feeding one per cent cholesterol with diets containing 12–15 per cent fat to guinea pigs results, in order, in the development of fatty livers rich in cholesterol, in enlarged spleens and in a severe anemia (1). Some of the erythrocytes are more and some less than normally resistant to breakdown by hypotonic salt solutions, a fact that suggests the presence of immature cells as well as an abnormally rapid rate of cellular breakdown.

Since the enlarged and hyperplastic spleens are developed only after the livers have been hypertrophic and fatty for some time there is a possibility that portal stasis may be responsible for the splenic enlargement and that hypertrophy of the spleen may in turn have caused destruction of the erythrocytes.

On the other hand the red cells of these animals have been found (2) to contain more cholesterol and less lecithin than normal cells. Hence destruction may also have been due to lipid imbalance within the cells.

The syndrome in the cholesterol-fed guinea pigs, i.e., anemia accompanied by both decreased and increased resistance of the erythrocytes to breakdown by hypotonic salt solutions, decrease in numbers of erythrocytes, increased bilirubin in the serum, deposition of iron in lungs and kidneys, pigment gall stones and hyperplasia of bone marrow, is very similar to that of one type of hemolytic anemia in man. Moreover, clinical cure of this anemia has frequently been brought about by splenectomy—presumably by decreasing the rate of destruction of the red blood cells. Splenectomy has also been reported to increase resistance of erythrocytes to destruction by hypotonic salt solutions in normal as well as in diseased animals. All of these findings indicated that a study of cholesterol feeding following splenectomy in guinea pigs might give information concerning the nature of the cholesterol anemia in this species.

**EXPERIMENTAL PROCEDURE.** Care of the animals and experimental procedures used in this laboratory have previously been described (1, 2).

<sup>1</sup> A preliminary report was published in the *Federation Proceedings* 5: 1, 1946.

<sup>2</sup> The data in this paper constitute part of a thesis submitted by Barbara Kennedy in partial fulfillment of the requirements for the degree of Doctor of Philosophy in animal nutrition, Graduate School, University of California.

The spleens were removed under aseptic conditions and with ether anesthesia from fifteen animals weighing between 400 and 500 grams. The animals were allowed a preliminary period for recovery on stock and/or basal diet<sup>3</sup> before transfer to the cholesterol diet. The time between splenectomy and the beginning of the cholesterol diet varied from 18 to 44 days.

Blood studies were begun three to four weeks after splenectomy. This eliminated the period of possible transient anemia which has been reported to be a frequent result of splenectomy (3). However, the severity of this anemia is much less than that following cholesterol feeding.

Lipids in blood and tissue, numbers of red blood cells, and hemoglobin values were determined by methods described in previous papers from this laboratory (1, 2). In addition, cell volumes were estimated using Van Allen hematocrit tubes. Average diameters of erythrocytes were determined by measuring 250 to 500 cells in blood smears stained with Wright's solution according to the method of Price-Jones.

Bilirubin determinations were made using the method of Malloy and Evelyn (5). Blood was obtained by means of heart puncture just before autopsy.

Frozen sections stained with Schlarlach R were used for study of lipid distribution in tissue, and formalin fixed tissues, embedded in celloidin and stained with potassium ferrocyanide and eosin, for demonstration of iron deposits.

**RESULTS AND DISCUSSION.** *Growth.* Following splenectomy the animals grew at normal rates (4 grams per day) when placed on the stock diet. Animals on the basal diet of higher fat content barely maintained their initial weight and most of the animals, after being placed on the one per cent cholesterol diet, lost weight rather steadily at average rates ranging between 0.9 and 4.6 grams per day. Signs of dehydration and extreme emaciation were not present during the periods studied.

*Blood changes.* A transient anemia has been observed (3) in normal animals following splenectomy. Cells have been reported to show signs of blood regeneration as judged by slight increases in reticulocytes, by the presence of red blood cells with refractive granules, and by occasional basophilic stippling and polychromasia.

Splenectomized guinea pigs on the *stock diet* gave no evidence of abnormal cellular breakdown (tables 1 and 2).

<sup>3</sup> Three different diets were used. 1. The *stock diet* was a commercial rabbit pellet (Globe A) furnishing protein, 15 per cent; fat, 2.5 per cent; crude fiber, 19 per cent; ash, 11 per cent, carbohydrate (by difference) 46.5 per cent. Total sterol (digitonin-precipitable material) was 0.06 per cent. 2. The *basal diet* contained commercial raw casein, 20 per cent; wheat bran, 6 per cent; dried brewer's yeast (Annheuser Busch, strain G), 10 per cent; Hubbel's salt mix, 2.5 per cent; hydrogenated fat, 12.5 per cent; cornstarch, 49 per cent. Total sterol was 0.10 per cent. 3. In the *cholesterol diet* one per cent added cholesterol replaced an equal weight of starch in the basal diet.

All of the animals received a weekly supplement of ascorbic acid, 15 mgm., in 18 cc. of fresh orange juice; "cerophyll", 2 grams; wheat germ oil, 9 drops; vitamin D, 1395 AOAC units (Delsterol); vitamin A, 770 IU (grey fish oil in corn oil).

Animals on the *basal diet* for about a week had red cell counts slightly elevated over the presplenectomy values. No unusual forms of erythrocytes were noted.

Animals on the *basal diet* for 70 to 90 days had somewhat lowered red cell counts (see table 1). The refractive granule red blood cells increased (up to 5 per cent) and other signs of blood regeneration, such as basophilic stippling and polychromasia, increased with the length of time on diet. Some of the cells were crenated by the Hayem's diluting fluid used for the red cell counts. A slight increase in the number of microcytes and macrocytes was shown by the animals on the basal diet. The serum bilirubin levels were somewhat increased (table 2).

TABLE 1

*Red blood cell counts on splenectomized guinea pigs on various diets*

NUMBER OF ANIMALS	BEFORE SPLENECTOMY	AFTER SPLENECTOMY			
	Stock diet, RBC millions	Days on special diet		RBC millions	Total days to count
2	5.1 (5.4, 4.8)		Stock	5.0 (4.9, 5.2)	59, 99
13*	5.3 (4.6-6.1)	9 (3-16)	Basal	5.7 (5.1-6.2)	15-42
6	5.3 (4.8-6.1)	78 (71-93)	Basal	4.6 (4.2-4.9)	84-128
2	5.4 (5.5, 5.4)	16, 17	Cholesterol	5.7 (5.6, 5.8)	51, 52
5	5.2 (4.6-5.7)	48-57	Cholesterol	3.2 (2.3-3.2)†	48-57

\* Same animals as in the groups below.

† With the exception of one animal that had a count of 5.0 and was probably dehydrated.

Splenectomized animals on the *1 per cent cholesterol diet* developed anemias the characteristics of which were not essentially different from those in the intact animals fed cholesterol. Crenation of all the red cells by the Hayem's diluting fluid was observed when the first counts were made twelve days after cholesterol feeding was begun. Numbers of cells were then normal. Red cell counts, hemoglobin, and hematocrit values decreased as cholesterol feeding was continued. Splenectomized guinea pigs took with one exception about fifty days for the red cell count to drop to three million. In animals with spleens the time varied from thirty to ninety days. The numbers of reticulocytes increased. After 48 to 57 days on cholesterol the reticulocyte counts of splenectomized animals averaged 16 per cent (range 4 to 27) as compared with an average of 1.3 per cent (range 0.3 to 2.4) after 15 to 27 days. The mean corpuscular volumes and the serum bili-

rubin (table 2) also increased. Fragility determinations made on two splenectomized animals showed some red cells with increased and some with decreased resistance to changes in salt concentrations. This agreed with Greaves' obser-

TABLE 2  
*Serum bilirubin in normal and splenectomized guinea pigs fed cholesterol*

DIET	DAYS ON DIET	NUMBER OF ANIMALS	SERUM BILIRUBIN		
			Total indirect bilirubin, mgm./100 cc.		Type of reaction
			Mean	Range	
Normal					
Stock.....		3	0.04	0.00-0.06	Indirect
Basal.....	76	4	0.13	Pooled sample	Indirect
Cholesterol 1%.....	54-95	8	1.56	0.23-5.19	Direct
Splenectomized					
Stock.....	43-48	3	0.00	0.0	
Basal.....	76-93	6	0.12	0.00-0.20	Direct
Cholesterol 1%.....	16	2	0.54	0.25-0.83	Direct
Cholesterol 1%.....	49-58	5	1.38	0.47-2.28	Direct

TABLE 3  
*Lipid content of livers and blood serum of splenectomized guinea pigs*

DIET	NUMBER OF ANIMALS	DAYS ON DIET, AVERAGE	CHANGE IN BODY WEIGHT, GRAMS PER DAY, AVERAGE	LIVER						SERUM
				Grams	% of body weight	Per cent of moist weight				Total cholesterol
						Fatty acid	Free cholesterol	Total cholesterol	Lecithin	
Stock	2	46 (43, 48)	+4.2 (3.2, 5.1)	19.6 (19.5, 19.6)	3.5 (3.1, 3.8)	4.4 (4.0, 4.9)	0.26 (0.24, 0.27)	0.30 (0.27, 0.33)	4.4 (4.2, 4.7)	mgm. % 88*
Basal	6	80 (71-93)	+0.5 (-0.1- +1.0)	18.4 (15.0-24.6)	3.9 (3.6-4.2)	7.3 (5.3-10.3)	0.30 (0.26-0.37)	0.44 (0.29-0.55)	3.5 (2.6-4.4)	90 (63-98)†
1% Cholesterol	2	16 (16, 17)	-4.6 (-4.2, -4.6)	23.4 (23.0, 23.9)	6.0 (5.6, 6.3)	8.4 (7.3, 9.6)	0.54 (0.53, 0.56)	1.55 (1.48, 1.62)	3.0 (2.9, 3.2)	
	5	56 (49-58)	-1.9 (-0.9- -4.6)	32.9 (24.3-37.8)	9.0 (7.4-11.4)	17.2 (12.8-20.4)	0.81 (0.79-0.89)	3.58 (2.32-4.49)	2.8 (2.0-3.2)	392 (272-636)

\* One animal.

† Three animals.

vations (1) on the cells of intact animals fed cholesterol. A limited series of observations on splenectomized animals also indicated an increase in average red cell diameter on the cholesterol diet. For instance, in one animal on stock diet the average cell diameter was found to be 7.4 microns; in two on the basal diet, 7.4 and 6.8 microns; while in one on the cholesterol diet, 8.7 microns.

*Tissue changes.* In animals on the *stock* diet all of the organs examined—liver, lung, kidney, adrenal, and bone marrow—appeared normal both grossly and microscopically. A few small hard gallstones were present in one of the two animals.

The animals on the *basal* diet of fat content identical with the cholesterol diet showed only slight tissue changes. The bone marrow and livers appeared normal. Weight of the livers as per cent of the total body weight (table 3) was slightly higher than for the animals on stock diet, probably due to the poor growth of the animals on the basal diet. Microscopically the livers had a slight accumulation of fat in the hepatic cells about the central veins. Small iron deposits were noted in the proximal convoluted tubules of the kidneys. A few gallstones were present in three of the six animals.

Guinea pigs which had been on *1 per cent cholesterol* for about fifty days presented pathological changes similar to those of the unsplenectomized animals on the same diet, i.e., hyperplastic bone marrow, enlarged pale livers which microscopically showed generalized fatty degeneration, iron deposits in the proximal convoluted tubules of the kidneys, lungs with thickened alveolar walls which contained macrophages laden with iron and fat. All five of the splenectomized animals fed cholesterol for about fifty days had gallstones which consisted of mucoid material containing a large number of hard and soft stones of varying sizes. Animals receiving cholesterol for only 16 or 17 days had no gallstones. Absolute weights of adrenals for animals on the three diets were the same (average 0.32 gram), but because of the weight loss this meant that adrenals constituted 0.057 per cent of body weight for the pigs on stock diet, 0.067 for the pigs on basal diet and 0.093 per cent for the animals on the cholesterol diet.

*Lipid content of liver and blood.* Slightly more fatty acid and free and total cholesterol were present in the liver when splenectomized guinea pigs were fed the basal diet than when they were fed the stock diet (table 3) presumably because of the higher fat intake. Liver lipids in cholesterol fed animals increased with the time of feeding. At 50 days the splenectomized animals had liver lipids similar to those of intact animals given the same diets (2). The percentage of lecithin in the liver progressively decreased with time of cholesterol feeding.

The hypercholesterolemia which accompanied cellular breakdown in these guinea pigs was greater than that reported after splenectomy in other species (3) in which it was supposed to explain the increased resistance of the erythrocytes to breakdown which followed removal of the organ (6).

#### SUMMARY

The reaction of fifteen splenectomized guinea pigs to control and cholesterol rich diets has been studied.

Growth of these animals was normal while they were on a stock ration but a semisynthetic diet containing 12.5 per cent fat which had given fair growth in intact animals failed to produce weight gains in the splenectomized group. Operated animals consistently lost weight when fed cholesterol.

There was no evidence of abnormal red cell destruction in the splenectomized

treated and KCl-treated animals the site, frequency and strength of stimulation have not been sufficiently varied to provide adequate results for critical analysis of the direct effects upon the muscle as compared with the indirect effects through the nerve and neuromuscular junction.

The purpose of this study is to investigate the tension and the time course of muscle responses to single and repetitive stimuli in normal rats, and in rats in which the K level of the muscle presumably has been altered gradually by adrenalectomy, DCA treatment and intraperitoneal injection of KCl. Both direct and indirect stimulation have been employed in an attempt to separate neuromuscular from purely muscular changes of response.

**METHODS.** Rats weighing about 100 grams were used in these experiments because it was found that severe adrenal cortex insufficiency developed more consistently in young than in mature animals. All rats were fed Purina dog chow *ad libitum*. Bilateral adrenalectomy was accomplished by a one-stage operation in which the adrenals together with all surrounding fat were removed through dorsolateral incisions. Weight loss and appearance of muscular weakness were used as criteria of acute adrenal cortex insufficiency. Experiments on rats in which post-mortem examination showed development of accessory cortical tissue were discarded. DCA in sesame oil was given in daily subcutaneous injections. Two per cent KCl was injected intraperitoneally after the animals were prepared for the recording of muscle responses. Curarization was obtained by intravenous injection of 0.01 cc. of intocostin per 100 grams of body weight. Light ether anesthesia was used in all of the experiments.

The *in situ triceps surae* of the rat was held at its proximal end by a steel pin which was passed through the femur at the level of the origin of the heads of the gastrocnemius. A fine steel S-hook tied into the distal muscle tendons and a steel wire with an interposed glass link connected the tendons of the muscles to the isometric lever which was arranged for optical recording on bromide paper moving at 96 mm. per second. The construction of the myograph follows the principle outlined by Eccles and Sherrington (1930). The magnification of movement ranged from 100 to 300 times. The single response series were recorded with a 1.5 cm. torsion lever arm and the tetanus series were recorded with a 0.5 cm. lever arm. The tensions developed were such that the muscle shortened about 0.1 to 0.2 mm. during single responses. Most of the figures show reproductions obtained by plotting points from the original myograms. Stimulation of the muscle was accomplished by passing current from a thyatron tube (G.T.) oscillator through the primary coil of a Harvard inductorium, the secondary coil output being carried to the preparation. The stimulator was adjusted to deliver desired frequencies ranging from 2 or 3 per second for single responses to 400 stimuli per second for tetanic stimulation. The strength of stimulation was controlled both by regulating the breakdown voltage on the thyatron tube and by fractionating the transformer output. For direct stimulation fine wires were attached to the two ends of the muscle. The stimulus strength was usually 6 to 13 times maximal. The strength of stimulation to the sciatic nerve for indirect stimulation was usually several times maximal.

Tension is expressed in grams per gram fresh muscle. The measurements as cited in this paper refer to resting tension plus developed tension. The resting tension was usually approximately 100 grams per gram muscle. The rising time was measured from the beginning of the upward deflection of the record to the peak. The half falling time as used in this paper is measured from the peak of the tension curve to the point where this curve has returned half way to the initial resting tension.

Certain definitions have been employed to simplify the presentation of the results and the discussion. In using the term *maximal response* it is assumed that all fibers of the muscle contract singly in response to a single stimulus. The term *supramaximal response* designates responses in which repetitive contractions of muscle fibers are thought to occur. The word "twitch" is enclosed in quotations when referring to responses of this sort. Because there is a possibility that end-to-end application of stimuli to uncured muscle may stimulate the muscle through its nerve endings the word "direct" used in this connection is enclosed in quotations.

**RESULTS.** 1. *Responses to single stimuli.* A comparison of results obtained in normal and adrenalectomized rats with  $6 \times$  maximal stimuli delivered by end-to-end stimulation at 2 per second is shown in figure 1. For the early responses from the adrenalectomized animals the peak tension was about 50 per cent higher than for the normal, the rising time was longer and the half falling time was shorter than in the normal animals. Treppe continued through the first 200 responses in normal rats during which time the peak tension increased 31 per cent. Thereafter the peak tension remained about constant in normal muscle. In the adrenalectomized rats treppe resulted in a rate of increase of peak tension approximately equal to the rate observed in normal animals during the initial 50 responses. However, this resulted in only 11 per cent increase of contraction height in the operated animals and there then followed a sharp decline of tension which continued progressively throughout the series of 400 responses (fig. 1A). Rising time and half falling time decreased during the period of treppe in the normal rats, then increased slightly and thereafter remained relatively constant up to the 400th response. The rising time and half falling time also decreased in the adrenalectomized rats during the early responses but they increased progressively from the 50th or earlier to the 400th response (fig. 1, B and C). Series of 400 responses at 2 per second were obtained from muscle of normal and adrenalectomized rats by stimulation through the sciatic nerve. The curves plotted from these series were essentially the same as those obtained by the "direct" stimulation described above.

The augmented peak tension and prolonged rising time shown by records from the muscle of the adrenalectomized rat following single "directly" applied shocks suggested that repetitive responses might be occurring (fig. 2, 1A). In an attempt to analyze the differences between responses of muscles from normal and adrenalectomized animals in response to single stimuli, a series of experiments were performed in which the sciatic nerve was stimulated about 2 cm. from the muscle. The form of the isometric curve resulting when a maximal

stimulus is thus applied to the nerve of a normal animal is reproduced in 1B of figure 2. The peak tension of such a normal muscle was not further augmented by increase of shock strength until the strength was increased to approximately  $100 \times$  maximal. At this time a further increase in "twitch" tension appeared (fig. 2, 2B). When the same procedure was followed with adrenalectomized

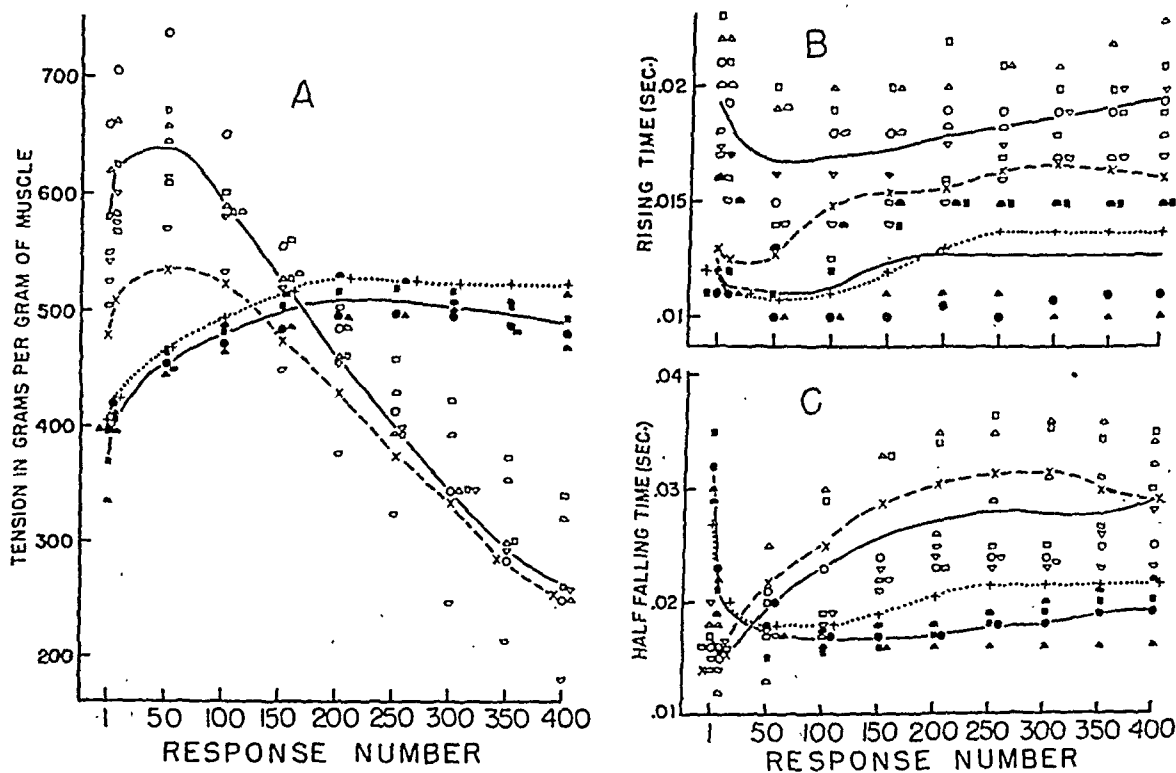


Fig. 1. A comparison in normal and adrenalectomized rats of the effect of end-to-end stimulation at 2 per sec. on the peak tension, rising time and half falling time during 400 responses. Solid symbols: normal; open symbols: adrenalectomized; solid lines: averages of the two groups. Dotted lines: a normal rat curarized; broken lines: an adrenalectomized rat curarized. The abscissas designate the responses in which measurements were made. A. The ordinates express the tension in grams per gram of muscle measured from zero tension to tension at the crest of the myograms. The resting tension was approximately 100 grams in all experiments used in this series. B. The ordinates express the rising time in seconds from the beginning to the crest of the myograms. C. The ordinates express the half falling time in seconds as measured from the crest to the point where the curve has returned half way to the original resting tension.

rats it was found that the threshold of excitability for the nerve was essentially unchanged in these animals. However, the determination of a true maximal stimulus strength was difficult. At a shock strength  $2.5 \times$  maximal for the sciatic nerve of the normal animal the record from the muscles of adrenalectomized animals showed a tension 150 per cent that of the maximal for normal muscle (fig. 2, 2B). Though the peak tensions and the rising times for normal maximal and the probably just maximal response from adrenalectomized rats

are essentially the same, the rate of relaxation is faster for the adrenalectomized animals (fig. 2, 1B). The same statement may be made for the supramaximal "twitch" responses from normal and adrenalectomized animals, respectively (fig. 2, 2B). The more rapid rate of response in 2B as compared with 1B of

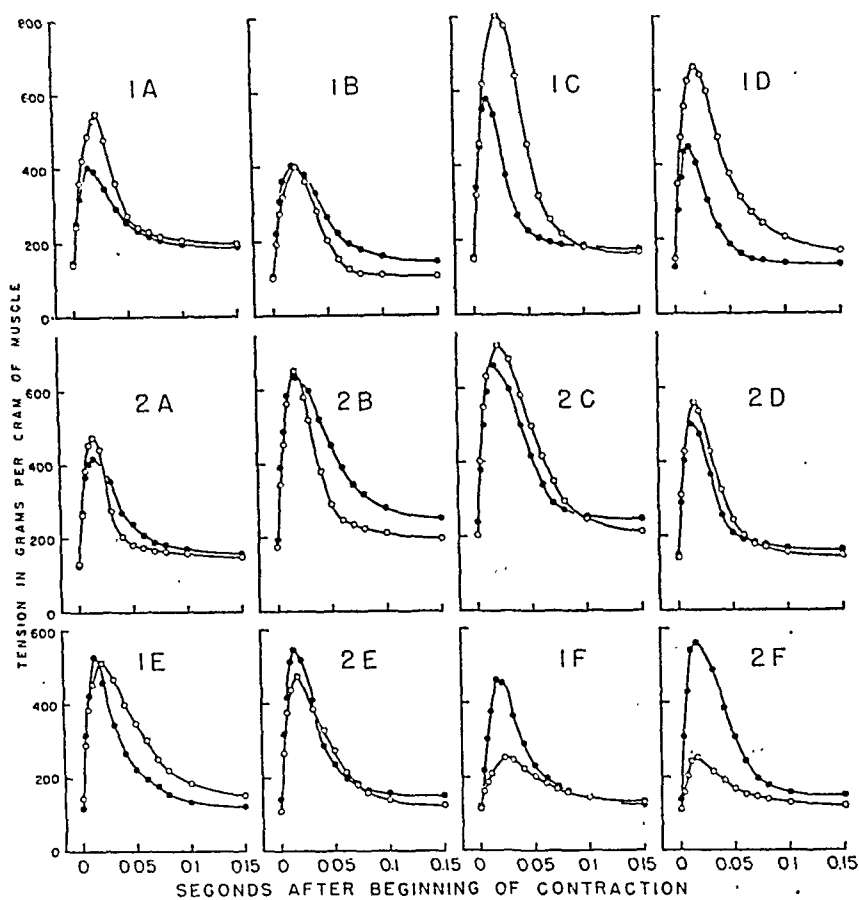


Fig. 2. Responses of the *triceps surae* to single stimuli at 2 per sec. Solid circles: responses from normal rats. Open circles: responses from adrenalectomized rats. 1A, 1C, 1D, 1E and 1F: uncured muscle stimulated directly with a shock strength  $6 \times$  maximal for normal muscle. 2A, 2C, 2D, 2E and 2F: curarized muscle stimulated directly with a shock strength  $13 \times$  maximal for uncured normal muscle. 1A and 2A: responses in rested muscle. 1C and 2C: post-tetanic responses after tetanus for 1 sec. at 400 per sec. 1D and 2D: response 50 in a series of 400 at 2 per sec. 1E and 2E: response 150 in a series of 400 at 2 per sec. 1F and 2F: response 400 in a series of 400 at 2 per sec. 1B. Responses to indirect stimulation with maximal shock strength for the nerve. 2B. Responses to indirect stimulation with shock strength for the nerve  $100 \times$  maximal in the normal rat and  $2.5 \times$  maximal in the adrenalectomized rat.

figure 2 is due to the warming-up process incident to the testing shocks employed to determine the supramaximal responses in the former. Action potential records of the early responses from muscle of adrenalectomized rats did not show a synchronized second response of the muscle fibers in the supramaximal contractions but the spread of the area of the action potential records indicated

that an asynchronous second response of at least a part of the fibers was occurring. The results of these experiments led to an attempt to determine whether these apparently repetitive responses could be obtained in curarized muscle.

It was found that a shock strength *ten* times that necessary to produce a maximal response by end-to-end stimulation of normal muscle was just sufficient for production of a maximal response to single shocks after curarization. Therefore, a shock strength  $13 \times$  maximal for normal muscle was used for stimulation of curarized muscle. The increase of shock strength required for maximal responses after curarization suggested that some of the "direct" stimulation of uncurarized muscle involved nerve terminals. However, the similarity of responses resulting from  $6 \times$  maximal stimulation in normal muscle (fig. 2, 1A) and  $13 \times$  maximal stimulation in curarized normal muscle (fig. 2, 2A) indicated that no repetitive impulses were being transmitted through the nerve terminals of the "directly" stimulated normal muscle. When adrenalectomized rats were curarized and stimulated directly with  $13 \times$  maximal stimuli the peak tension and rising time in rested muscle were significantly less than the peak tension and rising time in the uncurarized muscle of such animals receiving  $6 \times$  maximal stimulation (fig. 2, 1A and 2A). The marked effect of curare on peak tension and rising time in the unfatigued muscle of adrenalectomized animals is shown graphically in figure 1. Moreover, a moderate effect of curare in adrenalectomized rats was still apparent during the rapid decline of peak tensions occurring between the 50th and 400th responses (compare 1D to 1F with 2D to 2F of fig. 2). Completeness of curarization was proved by the failure of the muscles to respond to indirect stimulation either before or after the periods of direct stimulation.

When the initial responses of indirectly stimulated normal and DCA-treated muscles, respectively, are compared, one finds that the rising time, peak tension and half falling time (fig. 3, 1A) are markedly increased after treatment with large doses of DCA (2 mgm. per day for 45 days and thereafter 4 mgm. per day for 15 days). The augmentation of peak tension and prolongation of rising time for the DCA-treated muscles are even greater than that found in adrenalectomized animals and suggest that repetition is also a factor in these responses. The character of the changes found in the time course of the individual responses obtained during a series, however, indicates that the mechanism responsible for the repetition is not the same in the two groups of animals. The peak tension and rising time for responses from DCA-treated muscles diminished rapidly in responses subsequent to the first. The result of this was that even as early as the 6th response the peak tension had become less than and the rising time equal to the corresponding values for the response of normal muscle (fig. 3, 1B). The rate of relaxation of the 6th response was somewhat faster than that of the initial response but it was still much slower than the rate of relaxation in normal muscle.

In a series of 4 rats given 1.5 mgm. of DCA daily for 45 days, 400 end-to-end shocks through the muscle gave responses which showed treppe about equal to that of normal muscle without showing initial decline of tension like that

observed in rats given larger doses of DCA. Following the period of *treppe* the muscles from the DCA-treated animals did not show the severe progressive loss of tension which is characteristic of the adrenalectomized animal.

2. *Potentiating effect of tetanus and of KCl.* Following a brief period of tetanus a muscle may show potentiation of the response to a single shock. This occurs

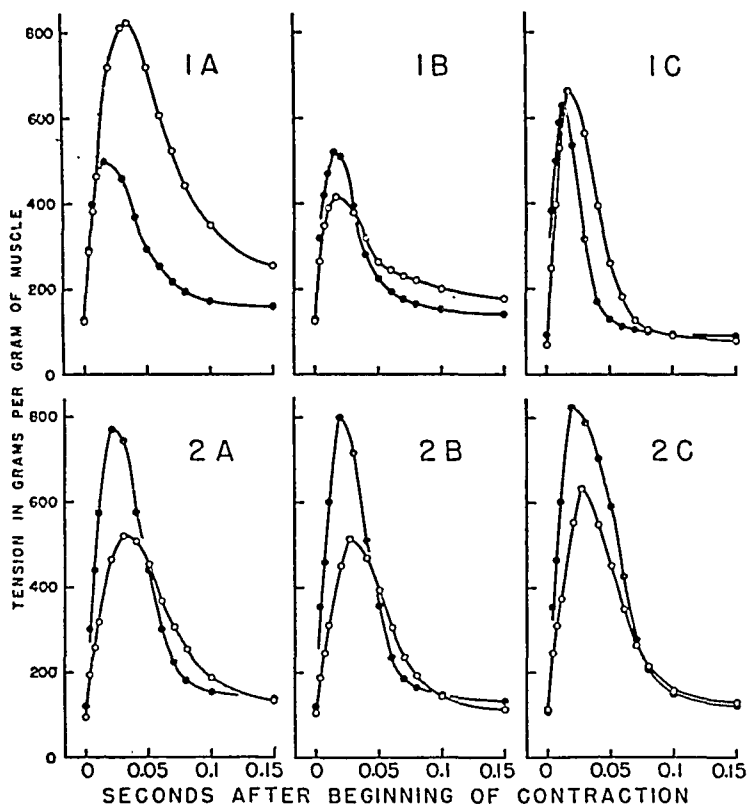


Fig. 3. Reproduction of myograms showing the effects of DCA and KCl on the response of muscle to single stimuli through the cut sciatic nerve. Open circles: responses from a rat given subcutaneous injection of 2 mgm. of DCA per day for 45 days and thereafter given 4 mgm. of DCA per day for 15 days. Solid circles: responses from a control rat. 1A, 1B and 1C: responses before KCl treatment. 2A, 2B and 2C: responses after intraperitoneal injection of 240 mgm. of KCl per 100 grams of body weight in the DCA-treated rat and 80 mgm. of KCl per 100 grams of body weight in the normal rat. 1A and 2A: response 1 in rested muscle. 1B and 2B: response 6 recorded immediately before tetanus. 1C and 2C: first response after tetanic stimulation at 225 per sec. for 1.5 sec.

in a similar manner for previously rested muscles of normal, adrenalectomized and DCA-treated rats (compare 1C with 1A in fig. 2 and compare 1C with 1B in fig. 3). Marked post-tetanic potentiation has been observed in muscle of normal and adrenalectomized rats with stimulus frequencies of tetanus varying from 30 to 130 per sec. (fig. 5). The potentiating effect of tetanus was not impaired in normal or adrenalectomized rats by complete curarization (compare 2C with 2A in fig. 2). The greatest potentiation which we have found occurred

after indirect tetanic stimulation in DCA-treated animals (compare 1C with 1B in fig. 3). In the DCA-treated rats the rising times in the post-tetanic and pre-tetanic responses were about equal.

The potentiating effect of intraperitoneally injected KCl (80 mgm. per 100 grams of body weight) on the tension of a response of normal rested muscle to a single indirect stimulus was striking (compare 2A with 1A in fig. 3). Because the pre-tetanic responses of KCl-treated animals developed abnormally high peak tensions the potentiating effect of tetanus was relatively less although the actual tensions of the post-tetanic responses were greater than those found in untreated normal animals (compare 2C with 2B in fig. 3). KCl treatment of curarized normal animals produced an increase of peak tension in response to single direct stimuli approximately 50 per cent as large as the increase found after KCl injection in uncurarized animals indirectly stimulated. However, the curarized muscle of normal animals treated with KCl showed marked post-tetanic potentiation. This effect of tetanus cannot be attributed to decurarization of the neuromuscular junction because no response of the muscle could be elicited by indirect stimulation immediately following the period of tetanus. Even when stimulation at a frequency of 225 per sec. was applied to the nerve there was no muscle response either during the burst or in response to a single shock also applied to the nerve one half second after termination of the burst.

The potentiating effect of KCl on DCA-treated rested muscle given single indirect stimuli was less than that found for normal animals (compare 2B with 1B in fig. 3). In fact, KCl injection of DCA-treated rats resulted in a decreased peak tension of responses early in a series (compare 2A with 1A in fig. 3). When the 6th response of DCA-treated animals was used as the point of reference, a pre-tetanic response was found which was less than that for normal animals given KCl, but a response following tetanus in the former group showed a greater tension *increase* than was seen in the latter group (compare 2C with 2B in fig. 3). However, the actual tension of the post-tetanic responses was greater in the latter group of animals.

During end-to-end stimulation of the muscle the effects of a period of exercise on the post-tetanic tension changes, where the tetanus immediately followed the exercise, were quite different in normal, in adrenalectomized and in DCA-treated animals (fig. 4A). Although the increase of peak tension appearing in the post-tetanic response of exercised normal muscle was much smaller than the increase seen in rested normal muscle the total tension in the former exceeded that of the latter (compare circles in fig. 4A with closed circles in fig. 2, 1A and 1C). After similar exercise no potentiating effect of tetanus was induced in the adrenalectomized animal and a diminution of tension appeared in post-tetanic responses of DCA-treated muscle (fig. 4A).

3. *Tetanus-twitch ratios.* The tetanus-twitch ratio is expressed as the relation of maximal tetanic tension to the peak tension of the single response immediately preceding the tetanus. The term "twitch" is used in this connection to designate the response to a single stimulus of whatever strength and it does not exclude the possibility that muscle fibers may respond repetitively to single stimuli.

The average tetanus-twitch ratio for 3 normal rested muscles was 4.25. The ratio was 2.39 for the muscle of 3 adrenalectomized animals. Complete curarization did not alter the ratio in normal muscle. On the other hand, for the muscles of 3 adrenalectomized rats given curare an average value of 3.95 was obtained,

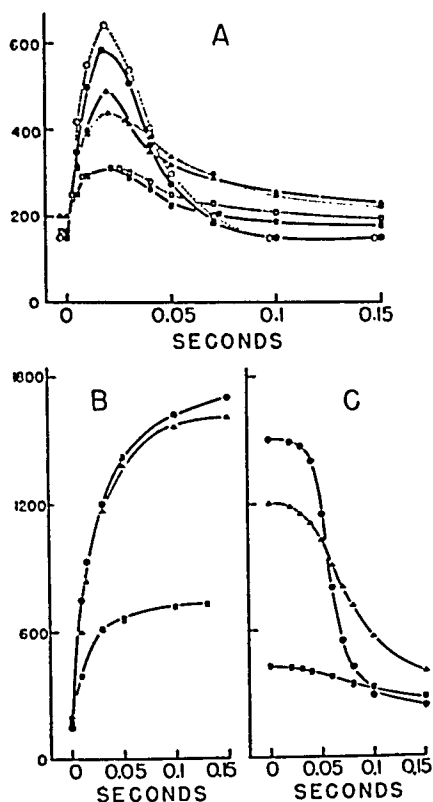


Fig. 4. Reproduction of myograms showing the effects of 400 end-to-end stimuli at 2 per sec. on the pre-tetanic and post-tetanic responses and on the rising phase and falling phase of tetanus in normal, adrenalectomized and DCA-treated rats. The ordinates designate tension in grams per gram of muscle. Subcutaneous injection of 1.5 mgm. of DCA per day was given for 45 days and thereafter 3 mgm. per day for 15 days.

A. Normal..... ● — ● pre-tetanic response; ○ ... ○ post-tetanic response.  
 Adrenalectomized... ■ — ■ pre-tetanic response; □ ... □ post-tetanic response.  
 DCA-treated..... ▲ — ▲ pre-tetanic response; △ ... △ post-tetanic response.

These responses are to be compared with responses in 1B and 1C of figure 3 and 1A and 1C of figure 2 which show pre-tetanic and post-tetanic responses in rested muscle. B. The rising phase of tetanic tension during stimulation at 225 per sec. in the same animals used in A. C. A continuation of the responses shown in B with the falling phase measured from the point of the last stimulus.

i.e., a figure approaching the ratio found for the normal muscle. KCl injection reduced the tetanus-twitch ratio markedly in normal muscle. Exercise in the form of 400 responses at 2 per sec. resulted in high tension in normal muscle and low tension accompanied by fatigue in muscle of adrenalectomized rats. Tetanus immediately following such exercise produced a low tetanus-twitch

ratio. It is worthy of note that regardless of the alteration of "twitch" height by the procedures in these experiments, the tetanus tension was essentially unchanged except in the previously exercised muscle of adrenalectomized animals in which it was reduced. Consequently variations in the tetanus-twitch ratio were, in the main, indications of variation in the "twitch" tension.

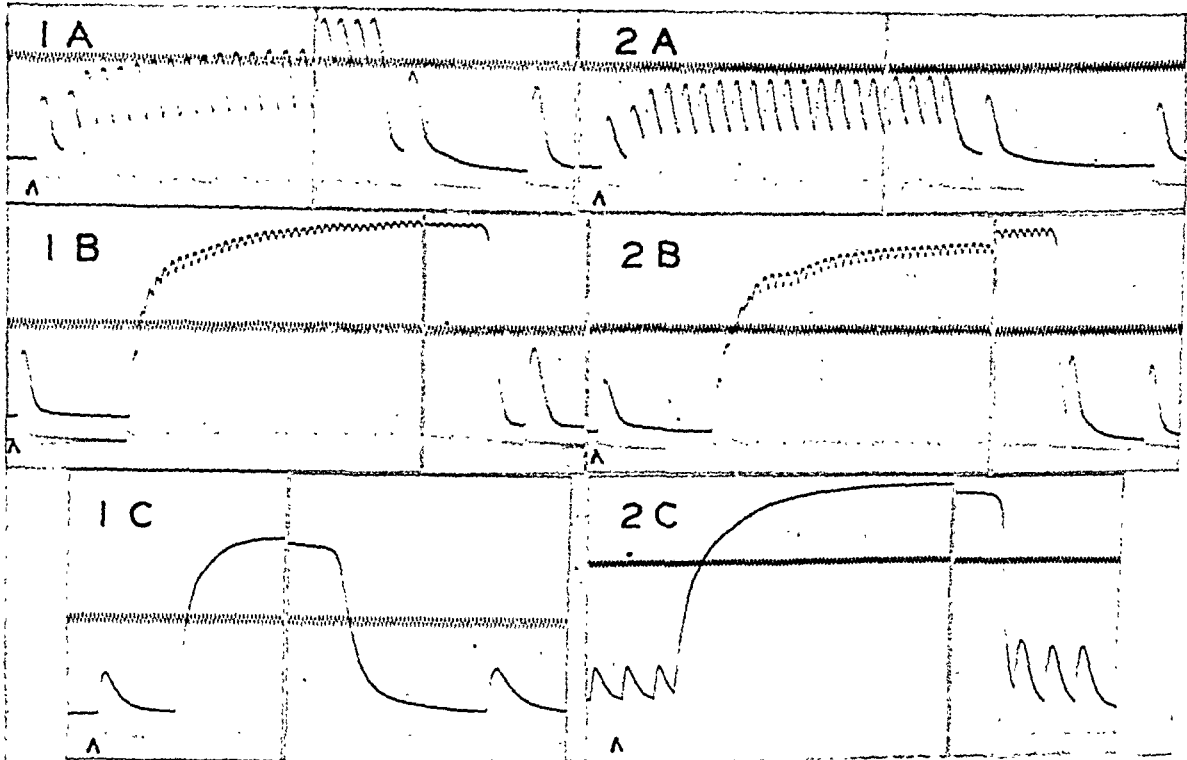


Fig. 5. Myograms of responses of the *triceps surae* in an adrenalectomized rat and in a normal rat showing the effect of different rates of stimulation on the development of tetanic tension and post-tetanic tension. 1A, 1B and 1C: responses from an adrenalectomized rat. 2A, 2B and 2C: responses from a normal rat. 1A and 2A: stimulation at 30 per sec. for 1.5 sec. 1B and 2B: stimulation at 65 per sec. for 1.5 sec. 1C: stimulation at 130 per sec. for 0.6 sec. 2C: stimulation at 130 per sec. for 1.2 sec. The points of the carets are placed at zero tension. Time is marked at 120 complete oscillations per sec.

4. *Responses to repetitive stimuli.* By varying the frequency of stimulation certain effects of adrenalectomy on the rate and extent of development of tetanic tension were demonstrated. Stimuli at 30 per second produced higher tension in the adrenalectomized than in the normal rat (fig. 5, 1A and 2A). At a frequency of 65 stimuli per second the tension developed in the adrenalectomized rat was only slightly higher than the tension attained in the normal animal (fig. 5, 1B and 2B). Furthermore, at the latter frequency the excursion of the individual responses diminished more rapidly in the operated animal as tetanic stimulation progressed. When the muscles were stimulated at 130 per second the tension curve developed by the muscle of the adrenalectomized rat was smoother throughout the period of stimulation (fig. 5, 1C and 2C). In normal

rat muscle stimulated end-to-end at 400 per second with  $6 \times$  maximal stimulus strength the maximal tension exceeded that attained in muscle of adrenalectomized rats about 20 per cent. The tension declined more slowly in the normal animals as tetanus progressed (fig. 6, 1C). The rising time of tetanus (400 per sec.) was approximately equal in both groups of animals, being somewhat longer in the normal muscle. The exercise resulting from 400 stimuli at 2 per second did not impair *maintenance* of tetanus tension in either normal or adrenalectomized rats but greatly reduced (40 per cent) the maximal tension attained in the adrenalectomized animals (fig. 6, 1D). The course of development and maintenance of tetanic tension in rested or exercised muscle of normal and adrenalectomized rats were not significantly altered by complete curarization (fig. 6, 2C and 2D). It is of interest to note in figures 6 and 4B that the rate of increase of tetanic tension during stimulation at high frequency was not greatly modified in the different types of experimental animals employed in this study: On the other hand, the rate of relaxation from tetanus was decreased both in adrenalectomized and in DCA-treated rats (fig. 4C).

When 225 stimuli per second were delivered through the nerve the tension declined faster in normal muscle than when direct stimulation was employed (compare solid lines and symbols in 1A and 2C of fig. 6). The more rapid rate of decline of tension appearing during indirect stimulation was regarded as a manifestation of Wedensky inhibition. The muscle in adrenalectomized rats stimulated indirectly at the above frequency lost tension less rapidly than did the indirectly stimulated normal muscle (fig. 6, 1A). The muscle of the normal animals was stimulated 400 times at 2.3 per second immediately before the tetanic stimulation at 225 per second. The same frequency of tetanic stimulation was delivered to the muscle of the adrenalectomized rats immediately after the peak tensions of the single responses had declined about 50 per cent, i.e., after approximately 300 responses at 2.3 per second. Although the 400 responses had caused no diminution of twitch tension in the normal muscles, the exercise resulted in an increased rate of decline of tension during the period of tetanus. For muscles of the adrenalectomized animals the effect of exercise upon the rate of decline of tetanic tension was less than in the normals (fig. 6, 2A).

When the sciatic nerve of a rat which had been treated with DCA (subcutaneous injection each day for 45 days with 1.5 mgm. doses and for 15 days thereafter with 3 mgm. doses) was stimulated at 225 shocks per second the beginning of decline of muscle tension occurred much earlier than in normal muscle and the rate of decline was greatly accelerated (compare solid lines and symbols in 1A and 1B of fig. 6). Such decline was not seen in directly stimulated muscles of DCA-treated animals. In a rat given 2 mgm. of DCA per day for 30 days the tension record of indirect stimulation at 225 per second made after intraperitoneal injection of KCl (160 mgm. per gram of body weight) showed a slower rate of tension decline than the record made in the same manner before KCl injection (fig. 6, 1B, solid and open circles). In another DCA-treated rat (2 mgm. per day for 45 days and 4 mgm. per day for 15 days) loss of

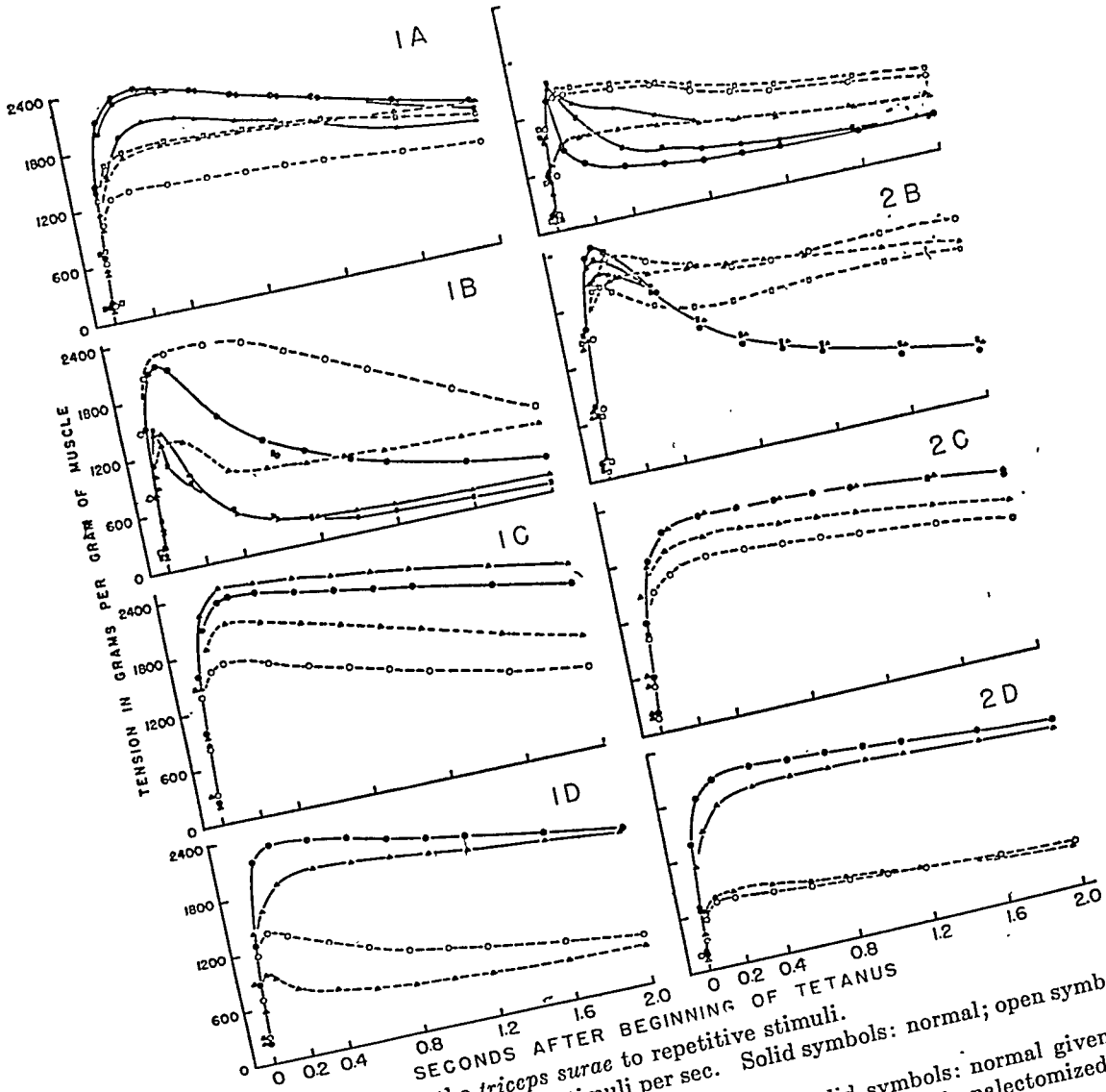


Fig. 6. Responses of the *triceps surae* to repetitive stimuli.

1A. Rested muscle, 225 indirect stimuli per sec. Solid symbols: normal; open symbols: adrenalectomized.

2A. Exercised muscle, 225 indirect stimuli per sec. immediately before tetanus. Solid symbols: normal; open symbols: adrenalectomized.

1B. DCA-treated rats, 225 indirect stimuli per sec. immediately before tetanus. Solid symbols: normal; open symbols: adrenalectomized.

2B. Normal rats, 300 indirect stimuli per sec. immediately before tetanus. Solid symbols: normal; open symbols: adrenalectomized.

1C. Before exercise, 225 indirect stimuli per sec. Solid symbols: normal; open symbols: adrenalectomized.

2C. Before exercise, 400 direct stimuli per sec. Solid symbols: normal; open symbols: adrenalectomized.

1D. Same treatment as in 1C except exercise (400 responses at 2 per sec.) was obtained immediately before tetanus. Solid symbols: normal; open symbols: adrenalectomized.

2D. Same treatment as in 2C except exercise (400 responses at 2 per sec.) was obtained immediately before tetanus. Solid symbols: normal; open symbols: adrenalectomized.

tension during indirect stimulation at 225 per second was partially relieved by injection of 250 mgm. of KCl per 100 grams of body weight (fig. 6, 1B, solid and open triangles). It seems worthy of notice that the decline of tension was most severe in animals receiving the larger doses of DCA. Furthermore, of the two DCA-treated animals given KCl the one treated with DCA for the longer period was relieved less.

Indirect stimulation at 300 per second in normal rats produced a rather sharp decline of muscle tension in the early part of the tetanus (fig. 6, 2B, solid symbols). A much slower decline of muscle tension occurred in these animals during similar stimulation 15 minutes after each had received 80 mgm. of KCl per 100 grams of body weight (fig. 6, 2B, open symbols). KCl was more effective in preventing loss of tetanus tension in normal than in DCA-treated rats (fig. 6, 1B and 2B). Furthermore, the dosage of KCl that produced marked changes in normal animals was ineffective in rats injected with DCA.

**DISCUSSION.** In attempting an analysis of these results consideration will be given to modifications of muscle response by various procedures (adrenalectomy, DCA treatment, KCl treatment and curarization) as they were observed in rested, exercised, post-tetanicly stimulated and tetanized muscle during direct and indirect stimulation.

*Rested muscle.* In rested normal muscle the response (fig. 2, 1B) to single indirect stimuli ranging from maximal to almost  $100 \times$  maximal shock strength was approximately equal to the maximal response (fig. 2, 2A) obtained by direct stimulation after curarization. Such responses to indirect stimulation are therefore regarded as true maximal responses produced by single contractions of all the fibers in the muscle. The increased peak tension and increased rising time, of the supramaximal response (fig. 2, 2B), which resulted from  $100 \times$  maximal indirect stimulation in the normal were due to asynchronous repetitive contraction of muscle fibers. The origin of this repetition in the nerve fibers or in the endplates of the muscle (presumably the former) was proved by the absence of supramaximal responses in curarized muscle stimulated directly. The marked increase of peak tension and rising time (fig. 2, 2B) produced by  $2.5 \times$  maximal indirect stimuli to rested muscle of adrenalectomized rats showed that the threshold for repetitive response was reduced by adrenalectomy. Thus at a stimulus strength  $2.5 \times$  maximal one may obtain, by indirect stimulation, maximal responses in normal muscle and supramaximal responses in muscle of adrenalectomized rats. The involvement of the nerve-motor endplate complex in the repetition observed in adrenalectomized animals was shown by the failure of supramaximal responses to appear after curarization (fig. 2, 2A).

Although intramuscular K is lowered by DCA treatment and increased by adrenalectomy the early responses both of rested DCA-treated muscle and of muscles from adrenalectomized animals showed supramaximal "twitch" tensions following the application of single indirect stimuli. However, the immediately following responses of the DCA-treated muscle differed from those seen in adrenalectomized animals in that they showed progressive decrease of peak tension so that by the 6th response the tension was usually even slightly lower than that to be expected for the normal muscle.

In rested KCl-treated muscle stimulated indirectly the increase of peak tension is probably due in part to repetition and in part to increased contractile strength of the muscle fibers. This view is based upon the observation that definite but smaller potentiation was obtained by KCl treatment after curarization. The reduction by curare of the amount of potentiation obtainable by KCl treatment appears to have been due to the blocking of repetitive impulses arising in the nerve complex of the muscle.

*Exercised muscle.* The treppe that appears when the rested normal muscle is stimulated at a rate of 2 per second results from an increase of contractile strength of the muscle fibers. The failure of curare to reduce the amount or duration of treppe showed that in these preparations neither repetitive contraction of muscle fibers nor recruitment of fibers involving the nerve-motor endplate complex plays a rôle in the staircase effect. The rate of increase of tension during treppe in muscles of adrenalectomized rats before and after curarization is equal to that of normal muscle but in operated animals the very early onset of fatigue results in the beginning of a then progressive diminution of peak tension (fig. 1A). The similarity of the rate and amount of treppe before and after curarization in adrenalectomized rats indicates that the increased tension produced by the staircase effect is independent of the repetitive contraction present in the uncurarized adrenalectomized animals. Because indirect stimulation, as compared with direct, did not increase the rate of decline of peak tension during continuation of exercise in adrenalectomized rats and therefore did not show an impairment of activity at the neuromuscular junction, the rapid decline of peak tension that occurred in curarized muscle of adrenalectomized animals is attributed to lessening of force of the muscle response.

*Post-tetanically stimulated muscle.* During direct and indirect stimulation of normal muscle the increase of twitch tension in post-tetanic responses was due to an increase of contractile strength of the muscle fibers since curare failed to reduce the amount of potentiation. The diminished rising time in responses of normal muscle after tetanus as compared with that of normal pre-tetanic responses provides additional evidence against the occurrence of repetitive contraction in the former. That recruitment of muscle fibers by improved neuromuscular transmission was not a factor in the post-tetanic potentiation was shown by the findings that this potentiation is the same in "directly" and indirectly stimulated normal muscle and directly stimulated curarized muscle while there is no response in indirectly stimulated curarized muscle. The potentiating effect of tetanus observed during complete curarization in our experiments is not in accord with the observations of Grumbach and Wilber (1940) on the isolated nerve-muscle preparations from the frog. These investigators found no post-tetanic potentiation after complete curarization in their preparations. The amount of post-tetanic potentiation produced in our experiments with the normal rat is of the same order of magnitude as that reported by Brown and von Euler (1938) for cat muscle. Since the tension ratios of pre- and post-tetanic "twitches" in the adrenalectomized animal are about the same with or without curarization, the potentiating effect of tetanus here, as in the

normal, must be mainly or entirely due to improvement of the contractile strength of the muscle fibers and not concerned with exaggeration of the repetition. In DCA-treated rats the similarity of the rising times in pre- and post-tetanic responses indicates that the potentiating effect of tetanus in these animals is produced by increased contractile strength of muscle fibers rather than by repetitive contraction of these fibers (fig. 3, 1C and 1B). In KCl-treated normal rats the diminished post-tetanic potentiation is doubtless due to the increase of contraction height already present in the pre-tetanic responses as a result of the KCl-treatment (fig. 3, 2B). The more pronounced potentiating effect of tetanus in DCA-treated animals injected with KCl may be explained by assuming that KCl treatment contributes a smaller increase of contractile strength to the pre-tetanic responses in these animals than in normal KCl-treated animals (fig. 3, 2B).

The diminished potentiating effect of tetanus immediately following 400 responses at 2 per second in muscle, "directly" stimulated, is a consequence of the increase of contractile strength induced by the exercise (fig. 4A, circles). The effect of exercise on the amount of post-tetanic potentiation that could be obtained in adrenalectomized rats was complicated by the effect of fatigue. After fatigue became evident in the muscle of these animals the potentiating effect of tetanus was inversely proportional to the extent of muscle fatigue, disappearing entirely after pronounced fatigue (fig. 4A, rectangles). As noted above marked potentiation was produced in rested muscle of adrenalectomized rats by 1 second of tetanic stimulation at 400 per second. Bjurstedt and von Euler (1939) found that post-tetanic potentiation was small or absent in adrenalectomized cats after 2 or 5 seconds of stimulation at 172 per second. Although only slight decline of twitch tension occurred in DCA-treated muscle during the 400 end-to-end stimuli which preceded tetanus, the diminished tension of the post-tetanic responses (fig. 4A, triangles) suggests that muscle weakness is produced by DCA treatment.

*Tetanized muscle.* Since maximal tetanic tension is essentially unchanged by KCl treatment and exercise in normal muscle and by adrenalectomy, it is concluded that the low tetanus-twitch ratio found is due to the high tension induced in the pre-tetanic responses by these procedures. The increased ratio found in adrenalectomized rats after curarization is a consequence of the abolition of repetition in the pre-tetanic response by the curare.

The ability of muscle in adrenalectomized rats to develop tetanic tension about equal to that attained in normal muscle in our experiments (fig. 6, 2C) is in agreement with the observations of Winter and Knowlton (1940). The marked decrease of tetanic tension after exercise in adrenalectomized animals is a result of the progressive failure of muscular activity that occurs during the period of exercise (fig. 6, 2D). The failure of curarization to diminish the development and maintenance of tetanic tension in rested or exercised muscle of normal and adrenalectomized rats proved that curare in the amounts used in these experiments does not impair the contractile strength of muscle.

During indirect stimulation of sufficient frequency to show considerable

Wedensky inhibition in normal muscle the *slower* decline of tetanic tension in adrenalectomized rats (fig. 6, 1A) suggested that K, which has been shown to be high in the muscle of such animals (Harrison and Darrow, 1938), may be facilitating transmission at the neuromuscular junction. This view is supported by experiments with DCA-treated animals (presumably having a low level of muscle K) in which tension decline was *faster* (fig. 6, 1B) than in normal animals stimulated at the same frequency. This interpretation is further supported by the observation that synaptic transmission is improved by perfusion of ganglia with KCl (Feldberg and Vartiainen, 1934). The relief of Wedensky inhibition by KCl treatment in normal and in DCA-treated rats (fig. 6, 1B and 2B) warrants the conclusion that K facilitates neuromuscular transmission.

#### SUMMARY

1. The responses of the muscle to single and repetitive stimulation through the muscle and to the nerve have been studied in normal, adrenalectomized, DCA-treated and KCl-treated rats.

2. Although the threshold of excitability for the nerve was approximately equal in normal and adrenalectomized rats, the shock strength required to produce supramaximal muscle responses to single indirect stimuli was much higher for the former than for the latter group of animals.

3. Single "direct" stimuli,  $6 \times$  maximal for the normal, produced in the rested muscle of adrenalectomized rats responses with a higher tension, longer rising time and shorter half falling time than those found in responses of normal muscle following similar stimulation.

4. Curarization did not alter the time course of the isometric responses to single direct stimuli in normal muscle but it reduced the peak tension and the rising time of responses to similar stimulation in muscle of adrenalectomized rats to values approximately equal to those for normal muscles. This effect of curare supports the view that responses obtained from uncurarized adrenalectomized animals by  $6 \times$  maximal "direct" stimulation involved repetitive contraction of muscle fibers.

5. Post-tetanic potentiation was not impaired by complete curarization. It is concluded that increased contractile strength of muscle fibers rather than repetitive contraction or recruitment of muscle fibers is responsible for the potentiating effect of tetanus in our experiments. Although post-tetanic potentiation in rested muscle of adrenalectomized rats was equal to that found in the normal, it was markedly reduced or absent in exercised muscle of operated animals.

6. The potentiating effect of KCl obtained during direct stimulation of curarized normal muscle was approximately 50 per cent of that attained by injection of KCl when the muscle was indirectly stimulated. It is suggested that potentiation appearing after KCl treatment of curarized muscle was a result of increased contractile strength of muscle fibers and that the reduction of potentiation by curare was due to block of repetitive impulses arising in the nerve complex of the muscle.

7. The marked exaggeration of rising time and peak tension observed during the first few muscle responses to single indirect stimuli in DCA-treated rats is indicative of prolonged repetition.

8. Diminution of peak tension in the responses of muscle from adrenalectomized animals progressed no more rapidly during nerve stimulation at 2 per second than during direct stimulation at the same frequency. Therefore, this aspect of fatigue cannot be attributed to neuromuscular failure.

9. The tetanic tension in unfatigued muscle was not significantly altered by the procedures used in this study; the changes of tetanus-twitch ratio are due largely to changes of twitch tension.

10. The maximal tetanic tension produced by direct stimulation was only slightly impaired by adrenalectomy. During indirect stimulation at 225 per second the early decline of tension was less rapid in adrenalectomized rats than in normal animals. Hence, after adrenalectomy neuromuscular conduction at high frequencies as well as low (vide 8) is at least as good as normal.

11. The Wedensky inhibition manifest in normal rats at high frequency of indirect stimulation was exaggerated by DCA treatment. KCl treatment diminished Wedensky inhibition in normal and in DCA-treated animals.

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# MODIFICATION OF RESPIRATORY MOVEMENTS BY VAGAL STIMULATION

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The mode of action of the vagal afferents from the lungs has been the cause of extensive research since the publication by Hering and Breuer (17) concerning the automatic vagal control of breathing. Methods of investigation of the so-called stretch reflexes of the lungs include observations on the respiratory responses to forced inflation and deflation of the lungs (1, 13, 17, etc.); recording of the electrical activity in the vagal afferents (1, 20, 21, 22, etc.); modification of conduction in the vagal afferent fibres by selective blocking (13, 14, 22), and various methods of stimulation of the central vagus (5, 12, 13, 14, 19, 22, 23, 26).

In earlier experiments on rabbits under urethane in which the respiratory responses to vagal stimulation were studied (23) it was concluded that two types of sensory fibres were probably involved. It was found that when the central vagus was stimulated with a low frequency stimulus, i.e., usually 20 per second or less, there was an increased respiratory rate and tonus, and when high stimulus frequencies were used, usually above 50 per second, respiratory inhibition with decreased tonus (resting lung volume) occurred. Substantially similar respiratory responses to vagal stimulation at different frequencies were subsequently reported by Wyss (26) and Gordh (12).

The evidence available leaves little doubt that more than one type of fibre may mediate respiratory reflexes from the lungs. The recent trend has been to emphasize the specificity of afferent pathways of sensation to the perceptive areas of the brain. Also there is apparently a high degree of specificity of many afferent pathways in the elicitation of particular reflex patterns. The concept that augmentor and inhibitory respiratory reflexes may be due to activation of different and more or less specific afferent pathways is therefore justified. However, the possibility also exists that different modifications of respiratory movements may occur, depending upon the *manner* in which a given reflex pathway is working. An important theoretical consideration is involved in such a possibility, since it would provide an additional mechanism by which sensory stimulation might evoke the various patterns of reflex response.

This report deals with further experiments on the respiratory reflexes incited by vagal stimulation. It offers an interpretation of the results which suggests that reflex control of respiration may depend not only upon the activation of

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various sensory pathways, but also upon a selective central response to activity in a given sensory pathway.

**METHODS.** Dogs were used mainly, and a few rabbits. Respiratory movements were recorded in most cases by means of a body plethysmograph, (in a few cases by means of a chest pneumograph) using light floats made of sheet aluminum of 5/1000 inch gauge. The plethysmograph records were therefore quantitative, and permitted observations on respiratory tonus. In many instances simultaneous femoral arterial blood pressure records were taken.

The total series of experiments is divided into 2 groups, depending on the anesthesia. Group 1 was given urethane 1.0 to 1.5 gram per kilo, intravenously or intraperitoneally. In some cases a small dose of morphine sulphate (1 to 2 mgm. per kilo) was added since it was previously found (16) that such doses of morphine do not affect the respiratory reflexes deleteriously. The second group was given sodium barbitone, 250 mgm. per kilo. Occasionally this was also supplemented with 1 to 2 mgm. per kilo of morphine, and the potentiating effect of morphine on this barbiturate in causing respiratory depression was observed (25).

The left vagus nerve was dissected out and divided low in the neck. In some instances both vagi were cut. At times, when the left vagus was ineffective on stimulation the right was used. After the recording equipment was connected the central end of the cut nerve was placed in a liquid type electrode. This consisted of two chambers side by side in a rubber block. A wick moistened with Locke's solution connected the nerve with a pool of Locke's solution in each chamber. Electrical connections with each chamber were made through a saline bridge to a saline calomel half-cell, which in turn connected by wire with one outlet of the stimulator. The electrode was covered with cellophane to prevent drying. Such electrodes worked consistently, i.e., with a constant response to a given stimulus for as long as 8 to 10 hours.

A detailed description of the characteristics of the stimulator used, and the manner in which the vagi were stimulated, is essential to the interpretation of the results. The stimulator was a thyratron type, similar to that described by Boyd (5). The intensity and frequency of its output could be independently controlled. The frequency range was from 3.3 to 325 per second. A feature of critical importance to the argument to be presented is that the wave form and duration of each shock were constant irrespective of the frequency of stimulation, as shown by visualization of the output on the cathode ray oscilloscope.

The stimulator also permitted stimulation for any desired duration up to 3 or 4 seconds, at any frequency. A given number of impulses could thus be transmitted to the brain. For example, if the frequency was set at 90, and the duration of stimulation set for 0.5 second, 45 shocks were automatically sent into the nerve. The number of shocks could thus be controlled by appropriately setting the frequency and duration dials, down to single shocks. The stimulator was calibrated in terms of known numbers of shocks for various set frequencies (e.g., frequencies of 60, 78, 90, 120 per sec.) by counts from records on moving paper.

Continuous vagal stimulation was obtained by throwing a toggle switch. Stimulation during either the inspiratory or expiratory phases alone was made possible by the use of light mercury switches, attached to the arm of the recording lever of the spirometer. These switches were operated in such a manner that the circuit was closed when the lever had moved only a few millimeters through the phase during which stimulation was to be applied. Thus an inspiratory stimulus was automatically applied throughout the whole inspiratory phase except the very start and carried over only into the beginning of the expiratory phase. A tracheal cannula was inserted in all cases and an effort made to estimate and duplicate as nearly as possible the normal dead space of the upper respiratory passages by adding a length of rubber tubing to the cannula where necessary.

TABLE 1

*Effect of continuous vagal stimulation. Urethane anesthesia composite results*

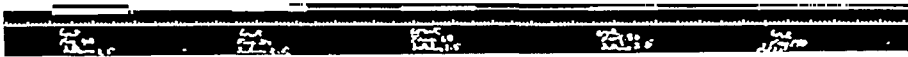
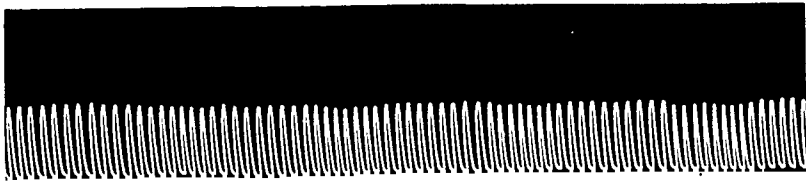
Number of experiments.....	29
Number of experiments showing resp. acceleration with low freq. stimulus.....	28
Number of experiments showing resp. inhibition with high freq. stimulus.....	29
Average low frequency stimulus.....	20 per sec.
Average % increase of resp. rate with low freq. stimulus.....	+34%
Average high freq. stimulus used.....	82.6 per sec.
Average % decrease of resp. rate with high freq. stimulus.....	-64%
Extremes of low frequency causing acceleration.....	3.3 to 60 per sec.
Average frequency of stimulus causing no change in resp. rate....	42 per sec.

NOTE: Measurements of respiratory rate during vagal stimulation are difficult due to changing respiration as CO<sub>2</sub> accumulates or is driven off. The method followed throughout was to determine the number of respiratory cycles during the first 15 seconds from the start of stimulation and to convert this to rate per minute.

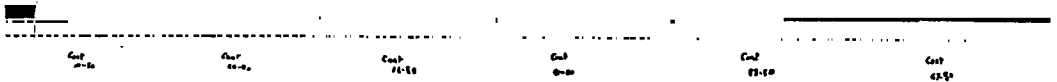
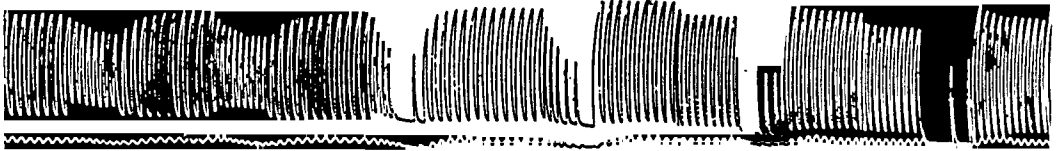
*a. Results of Vagal Stimulation Under Urethane.* Experiments were done on about 60 dogs and 8 rabbits. In this report most significance is attached to the results on dogs as the series of rabbits is small. It is worth noting however that the results obtained on rabbits were similar to those obtained on dogs. Of the dog experiments 48 were considered significant, the remainder being of no value owing to a variable and irregular respiration or to accidental death of the animal before complete results were obtained.

*Continuous vagal stimulation.* Table 1 demonstrates the composite effects of continuous vagal stimulation at various frequencies. (In each experiment the shock voltage was kept the same for all frequencies.) The table shows that what are called low frequencies caused respiratory acceleration in all instances except one and high frequencies caused respiratory slowing or arrest in all cases (figs. 1, 2, 3). In some animals acceleration resulted only with a very low frequency, e.g., 3.3 per second and in others it occurred even with relatively high frequencies, e.g., 41 per second. Acceleration resulting from low frequency stimulation was mainly due to shortening of the expiratory phase, with an earlier onset of

inspiration (fig. 2). In cases where a very low frequency was required to cause acceleration inhibition resulted at stimulus frequencies low in comparison with



A



B

Fig. 1 A. Dog. Urethane. Continuous stimulus, constant intensity. Frequencies of 10, 15, 20, 30, 40, 50 and 60 cause respiratory acceleration. Frequencies of 80 and 130 cause slowing.

Fig. 1 B. Dog. Urethane. Continuous stimulus, constant intensity. Frequencies of 10 and 20 cause respiratory acceleration. Frequencies of 36 and 41 cause first acceleration going over into slowing. Frequencies of 55 and 67 cause only slowing.

Note: In this and subsequent records inspiration is shown by upward movement on respiratory tracing. Time is indicated in one second intervals (small marks) and five second intervals (larger marks) on bottom line. Stimulus is indicated above or below the time line.

those usually required to cause inhibition. These results are in accord with those previously obtained on rabbits under urethane anesthesia (23) and with results

described by Gordh (12) and Wyss (26) and indicate that within limits a respiratory acceleration or slowing can be obtained by varying the frequency alone of afferent vagal stimulation.

Table 2 shows representative results of the effect of increasing the stimulus frequency with constant intensity. In this animal acceleration occurred with all frequencies used up to 41 per second, and slowing occurred with all frequencies used above 78. The zone where no change occurs is somewhat higher than usual.

That these results depend upon the frequency, rather than intensity of the stimulus is shown by experiments in which the intensity was increased while the

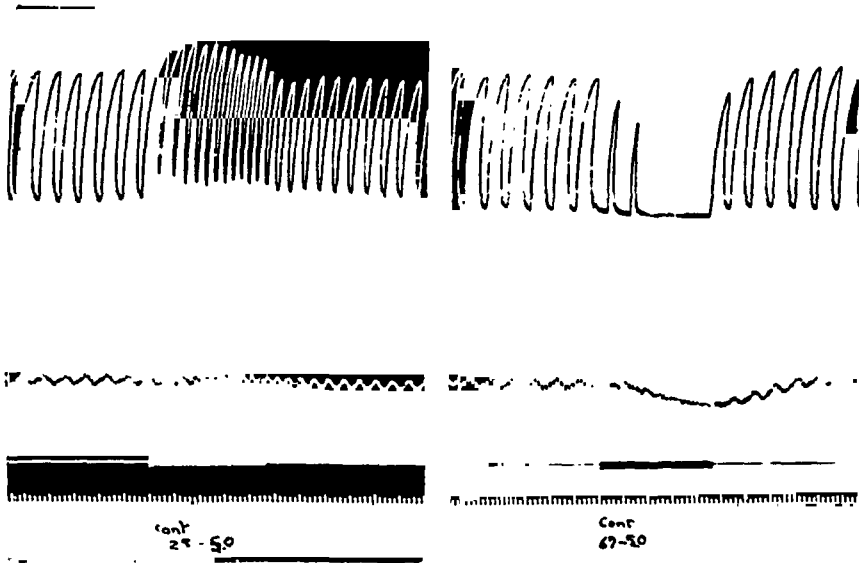


Fig. 2. Dog. Urethane. Intensity, 5.0. Continuous stimulus.

Left. Stimulus at frequency of 28 per second caused acceleration. The early onset of inspiration and reduced expiratory phases are evident.

Right. Frequency of 67 causes slowing of respiration. Prolonged expiratory phases are evident.

frequency was held constant. A more marked effect resulted with a stronger stimulus, but it was always an acceleration if the frequency was low, and a slowing if the frequency was high.

*b. Afferent vagal stimulation during the expiratory phase only. (Expiratory vagal stimulation.)* Table 3 shows representative results obtained with expiratory vagal stimulation at various frequencies. Again in each particular experiment the stimulus intensity was constant. The results on the whole are similar to those obtained with continuous vagal stimulation though perhaps less marked acceleration with low frequencies occurred. The significant feature of stimulation during expiration alone is that the acceleratory mechanism due to intermittent inspiratory inhibition is eliminated and the effect of low frequency afferent vagal stimuli causing the earlier start of each respiratory cycle (seen also with continuous stimulation (fig. 2)) becomes clear cut. The respiratory centre under the

influence of such stimulation is made to discharge sooner than it otherwise would and breathing is accelerated.

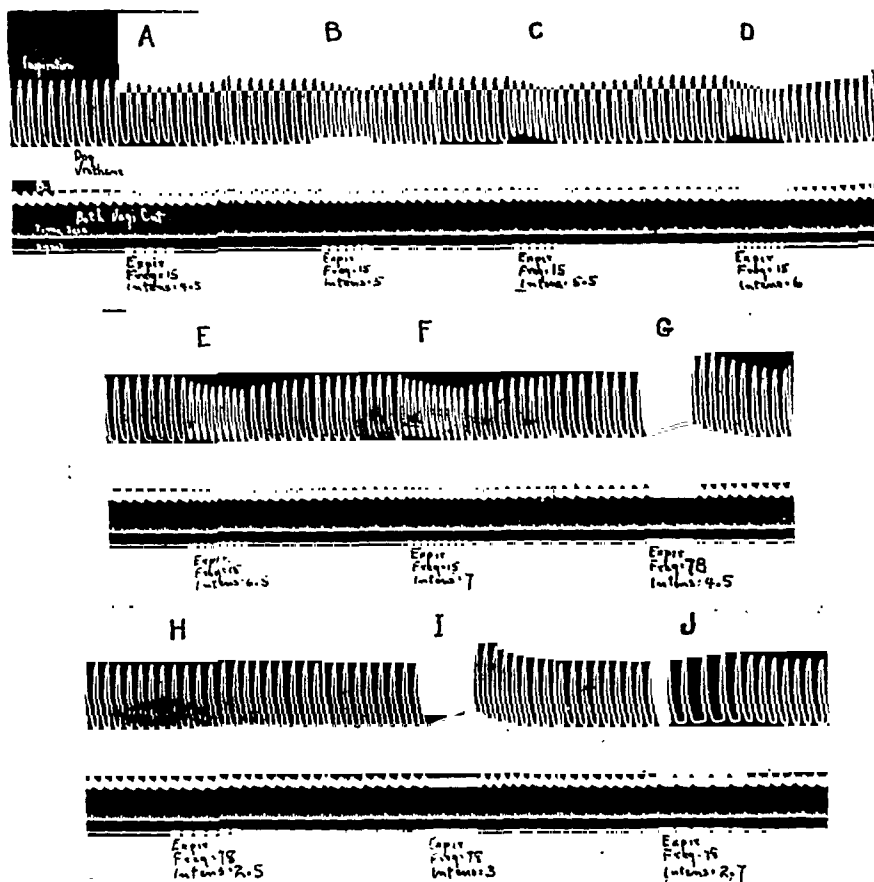


Fig. 3. Dog. Urethane. Expiratory stimulation A, B, C, D, E, and F, frequency of stimulus, 15. Intensity dial settings: 4.5; 5.0; 5.5; 6.0; 6.5; 7.0 respectively. More marked acceleration occurred with increasing intensity.

G, H, I, J, frequency of stimulus 78.

Intensity dial settings 4.5; 2.5; 3.0; 2.7 respectively. More marked slowing occurred with increasing intensity.

Note: Accurate assessment of the stimulus voltage at the nerve is difficult with electrodes of the liquid type used. The intensity of the stimulus cannot be stated in absolute values of voltage or current. But since the interelectrode resistance was high (<100,000 ohms) relative to the resistance of the linear voltage divider across the output of the stimulator (intensity control 10,000 ohms) the current drawn through the electrode systems was small. Intensity dial settings should therefore represent a linear voltage relationship.

Intensity dial settings were selected on the basis of observed physiological responses to stimulation.

High frequency expiratory vagal stimulation on the other hand caused a delay in the onset of each succeeding respiratory cycle, and thus slowed the breathing, apparently owing to inhibition of the respiratory centre. Figure 3 illustrates that these effects are due to the frequency rather than the intensity of the stimulus.

Increasing the intensity augmented the effectiveness of a stimulus but did not alter the type of response.

c. *Affrent vagal stimulation during the inspiratory phase only.* (*Inspiratory vagal stimulation.*) Table 3 shows that vagal stimuli which are effective in slowing the respiration when applied during expiration cause a marked respiratory

TABLE 2

*Effect on respiratory rate of progressive increase of stimulus frequency with constant intensity. Continuous stimulus. Dog. Urethane anesthesia*

STIMULUS FREQUENCY	RESP. RATE PRIOR TO STIMULUS	RESP. RATE DURING STIMULUS	% CHANGE IN RATE
15	28.2	34.8	+23.6%
22	31.2	39.6	+27.0%
30	29.4	40.8	+39.0%
43	34.8	45.0	+29.0%
78	36.0	22.8	-36.6%
92	25.0	8.0	-68.0%
130	22.8	0	-100.0%

TABLE 3

*Typical effects of vagal stimulation during inspiratory and expiratory phases. Urethane anesthetic*

EXPERIMENT	STIMULUS FREQUENCY	CHANGE IN RATE WITH EXPIRATORY STIMULUS	CHANGE IN RATE WITH INSPIRATORY STIMULUS
Rabbit.....	20	+17%	+3%
	162	-51%	+47%
Dog 1.....	20	+27%	+40%
	90	-69%	+142%
Dog 2.....	20	+29%	
	90	Arrest	+91%
Dog 27.....	18	+7%	
	78	-27%	+100%
Dog 29.....	18	+17%	+4%
	78	-25%	+70%
	108	-58%	

acceleration when applied during inspiration. Each inspiratory phase is reduced in both duration and depth and the subsequent expiratory phase is also shortened, a marked acceleration being the net result. As table 3 shows, even low frequency shocks are effective in producing respiratory acceleration when applied during the inspiratory phase. But the effect is slight compared with high frequency shocks from the point of view of both the acceleration and the reduction of tidal volume.

*Effect of Vagal Stimulation with Barbitone Anesthesia.* Experiments were performed on 8 dogs under soluble barbitone anesthesia (tables 4 and 5). Six of these dogs failed to show respiratory acceleration with low frequency stimuli (fig 4A and B). Two dogs did show an accelerator response and it is interesting to note that these results were obtained in animals which were lightly anesthetized, the usual dose of anesthetic having failed to produce complete anesthesia. In one of these animals (no. 39) increasing the dose of barbitone converted an accelerator response with low frequency stimulation to an inhibitory

TABLE 4  
*Effect of continuous vagal stimulation. Barbitone anesthesia*

EXPERIMENT	STIMULUS FREQUENCY	RESULTING CHANGE IN RESPIRATORY RATE
37	6.5 40	-64% Arrest
38	3.3	Arrest
39	10 41	+56% Arrest
41	10	Arrest
42	3.3 5.5 10	-41% -71% Arrest
43	3.3 5.5 10 20 41	+78% +66% +54% -12% Arrest
44	6 30 78	-25% -36% Arrest
45	3.4 22	-20% Arrest

response. Six of these animals however showed a marked respiratory acceleration with inspiratory stimuli (table 5) (fig.4B). One (no. 38) showed slowing even with this type of shock, indicating an inhibitory effect from each stimulus of sufficient degree and duration not only to cut short the inspiratory movement during which it was applied, but to delay the onset of the next inspiration.

*Effect of Limiting the Number of Shocks at an Inhibitory (High) Frequency.* In order to determine whether the respiratory responses to high and low rates of vagal stimulation depended upon the actual frequency of the stimulus, a series of experiments under urethane anesthesia was performed in which in each experiment

a particular frequency was employed, but the number of shocks at that frequency was varied. Having found a frequency and intensity of stimulus which caused

TABLE 5

*Effect of vagal stimulation during inspiratory and expiratory phases. Barbitone anesthesia*

EXPERIMENT	STIMULUS FREQUENCY	CHANGE IN RATE WITH EXPIRATORY STIMULUS		CHANGE IN RATE WITH INSPIRATORY STIMULUS	
		Per minute	%	Per minute	%
37	3.3	Arrest	-100	24 to 52	+113
	20	Arrest	-100		
	41	Arrest	-100		
	60	Arrest	-100		
	90	Arrest	-100		
38	3.3	Arrest	-100	52 to 44	-15
	90	Arrest	-100		
39	3.3	14.5 to 18.5	+28	16 to 18.5	+15
	10	15 to 16	+6.2		
	20	17 to 12	-14		
	41	19 to arrest	-100		
	90	.....	.....	16 to 20	+25
	.....	.....	.....	14 to 25	+79
	With deeper anesthesia		.....	12 to 20	+67
	3.3	15 to 16	+6.6		
	10	14 to 6	-57		
	20	16 to 4	-75		
	With still deeper anesthesia		.....		
	3.3	12 to 4	-67		
	10	12 to arrest	-100		
	90	.....	.....		
40	3.3	12 to 6	-50	13 to 17	+31
	10	9 to 2	-78		
	90	15 to arrest	-100		
41	3.3	6.5 to 2	-69	8 to 16	+100
	10	6.5 to arrest	-100		
	90	.....	.....		
42	3.3	21 to 12	-43	17.5 to 19.5	+11
	5.5	18 to 3	-84		
	10	18 to arrest	-100		
	90	.....	.....	18 to 20	+11
43	5.5	21 to 30	+43	13 to 22.5	+73
	10	19 to 24	+26		
	20	19 to 18.5	-2.6		
	41	19 to 6	-68		

marked respiratory inhibition when applied throughout the expiratory phase, the same frequency and intensity was subsequently used, but the duration of stimu-

lation was limited to a short fraction of the expiratory phase, and the number of shocks thereby restricted. In this manner 10 or 20 shocks, for example, might be applied in mid-expiration at a frequency of 90 per second. Usually the stimulus switch on the arm of the respiratory recorder was adjusted so that the shocks were applied as the respiratory recording lever approached the resting level,

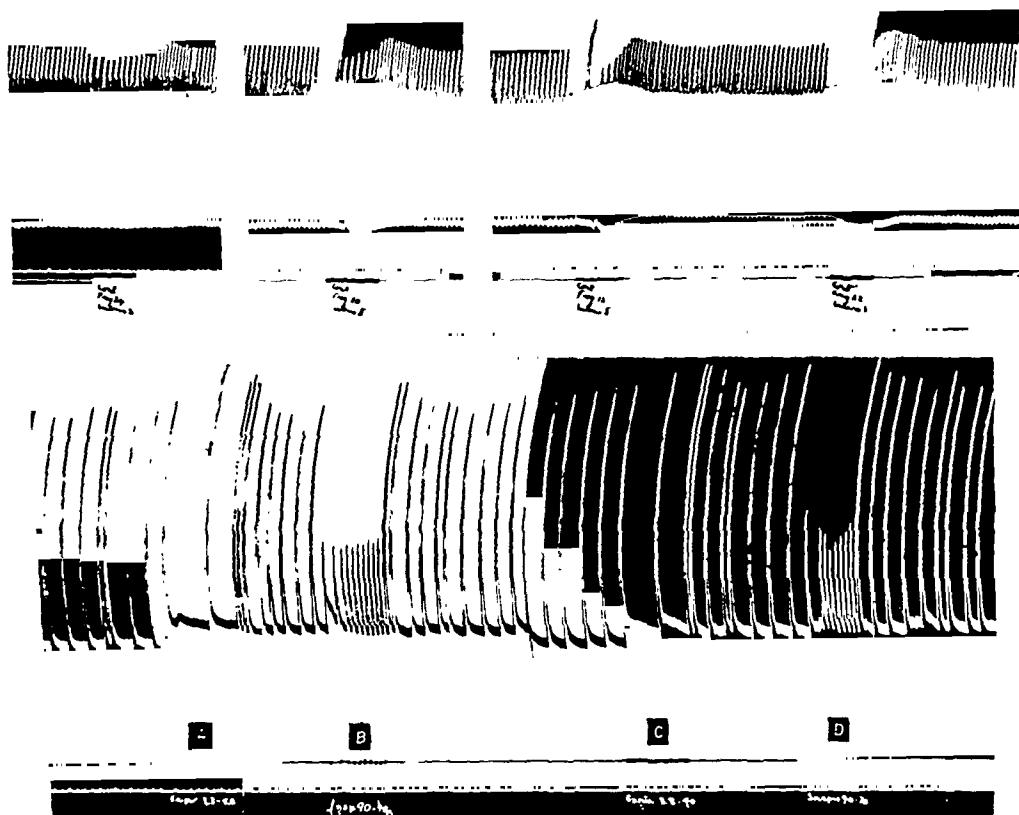


Fig. 4 A. (Upper) Dog. Soluble barbitone anesthesia. Continuous stimulus at constant intensity.

Frequencies of 3.4, 10, 15, and 22 cause respiratory slowing.

Fig. 4 B. (Lower) Dog. Soluble barbitone anesthesia. Expiratory stimulation at frequency of 3.3 per second causes respiratory slowing. (At A and C) Inspiratory stimulation with inhibitory stimulus, frequency 90, causes acceleration. (At B and D)

i.e., roughly in the middle of the expiratory phase. In some instances the switch was adjusted or worked manually so as to apply the shocks early or late in the expiratory phase.

Representative data are shown in table 6. As would be expected, in view of results from stimulation throughout the expiratory phase, if the duration of the stimulus was long enough (i.e., the number of shocks great) respiratory slowing invariably occurred. But when the number of shocks was restricted to a low

enough figure, even though the frequency was not changed; respiratory acceleration occurred. This acceleration was less marked than that resulting from low frequency stimulation, but it was nevertheless definite (fig. 5). From records

TABLE 6

*Effect of limiting the number of shocks. Central vagus. High frequency stimuli in expiration*

EXPERIMENT	STIMULUS FREQUENCY	NUMBER OF SHOCKS	NORMAL RESP. RATE PER MIN.	RESP. RATE WITH STIMULATION	PER CENT CHANGE IN RESP. RATE
3	90	2	17.4	21.6	+24%
		5	16.8	17.4	+3.6%
		10	16.8	14.1	-16%
		20	16.8	12.6	-25%
4	90	10	14.4	15.6	+8.3%
		40	15	13.2	-12%
6	60	4	22.8	24.0	+5.3%
		12	22.2	20.4	-8.1
15	78	1	23.2	22.4	-3.5%
		5	25.2	23.6	-6.3%
17	78	7	23	27.2	+18%
		12	23.2	24	+3.4%
		19	24	20.5	-14.5%
		36	24	17.6	-27%
19	78	8	24.6	29.0	+18%
		38	23.8	23.0	-3.4%
20	130	8	26	33.6	+29%
		37	26.4	24	-9.1%
		46	26.8	22.4	-16%
		98	26.4	18.6	-30%
23	90	20	10.5	12.3	+17%
		40	10.5	9.9	-5.7%
46	78	14	19.8	21.6	+9%
		43	19.8	18.6	-6%
47	130	5	28.8	31.6	+9.7%
		23	28	24.8	-11%
48	90	40*	11.3	13.3	+18%

\* This was the largest number of shocks at a high frequency that caused acceleration.

on rapidly moving paper this acceleration was shown to be due to a reduction in the duration of the expiratory phase, resulting in a definite reduction in the duration of each complete respiratory cycle (from inspiration to inspiration) (fig. 6.

table 7). There were times when no acceleration occurred even with as few as 3 or 4 shocks or even a single shock (expt. 15—table 6) and such instances were frequently associated with a poor accelerator response to low frequency stimuli. In almost all instances a number of shocks could be determined intermediate between those causing acceleration and slowing which resulted in no change in respiratory rate.

TABLE 7

*Effect of brief volleys at rapid frequency on the duration of the phases of the respiratory cycle.  
Expiratory stimuli*

STIMULUS FREQUENCY	NUMBER OF SHOCKS	DURATION OF INSPIR.		DURATION OF EXPIR.		DURATION TOTAL CYCLE	
		Normal	With stim.	Normal	With stim.	Normal	With stim.
		seconds	seconds	seconds	seconds	seconds	seconds
90	40*	3.0	3.0	2.3	1.5	5.3	4.5
90	5	2.0	2.0	1.6	1.0	3.6	3.0
78	27	1.2	1.2	1.9	1.5	3.1	2.7
78	2	2.2	2.2	1.6	1.3	3.8	3.5

\* See footnote to table 6.

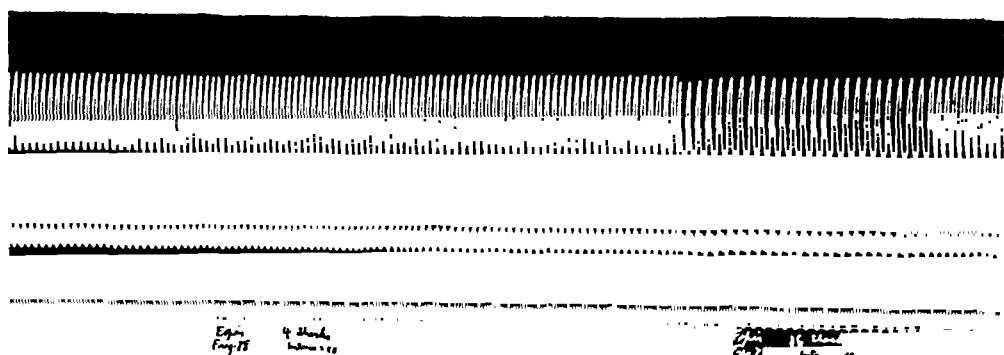


Fig. 5. Dog. Urethane.

Left. 4 shocks applied in mid-expiration at a frequency of 78 per second cause acceleration of respiration. Normal respiratory rate 24 per min. Rate during stimulus, 28 per min.

Right. 36 shocks applied in mid-expiration at frequency of 78 per second cause respiratory slowing. Normal respiratory rate 24 per minute. Rate during stimulus, 17.4 per minute.

Some further observations pertinent to the discussion to follow were also made. If a stimulus was employed which was of high frequency and long duration, and which would thus delay the onset of the next inspiration if applied in mid expiration, this same stimulus sometimes failed to delay the inspiration about to occur if it were applied very late in the expiratory phase, i.e., a fraction of a second before the expected inspiratory movement. When so timed by manual use of the switch, it frequently precipitated a sharp and slightly premature inspiration

(fig. 7). The explanation for this phenomenon appears to be that the first few impulses to reach the centre threw it into activity before the inhibitory influence

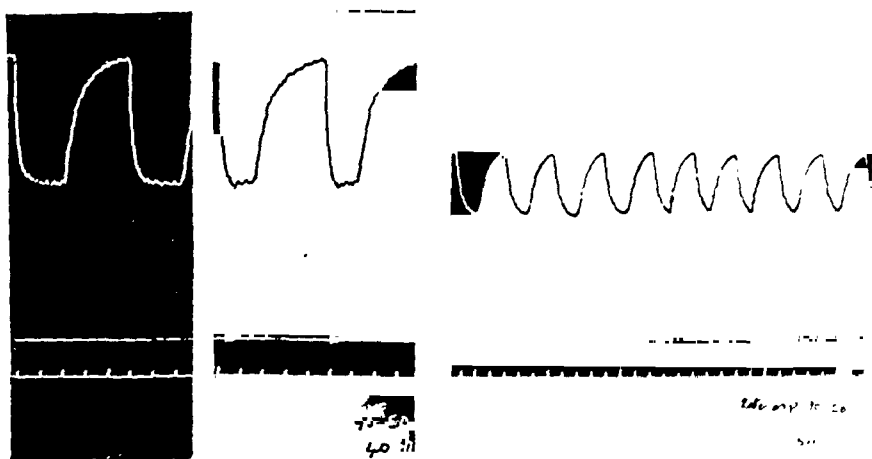


Fig. 6. Dogs. Urethane. Rapid kymograph records showing that acceleration resulting from a limited number of shocks in expiration is due to the inciting of an earlier inspiration, with reduced duration of expiratory phase. Normal curves at left; effect of stimulus (indicated above time lines) shown at right.

A. (Left) 40 shocks, at frequency of 90 (Intensity setting of 5.0)

B. (Right) 5 shocks at frequency of 90. (Intensity setting 5.0)

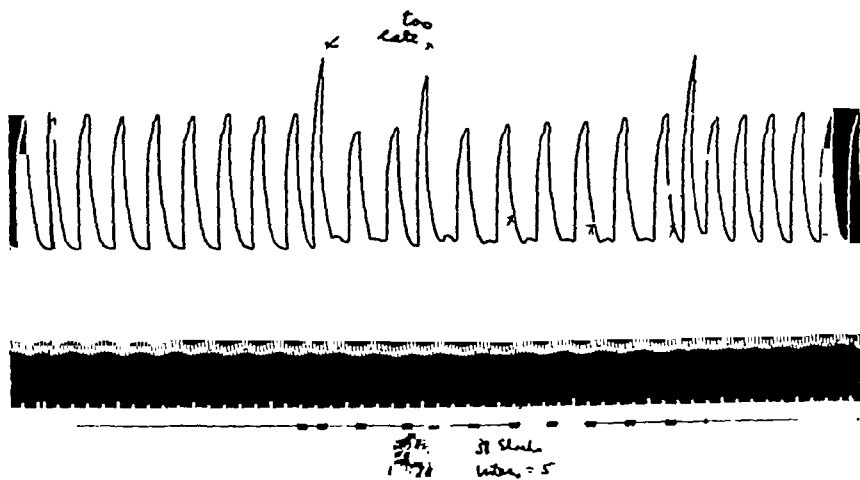


Fig. 7. Dog. Urethane. Stimulus switch worked manually; 38 shocks at frequency of 78 applied in mid-expiration usually delayed the next inspiration. When applied too late in expiratory phase the same stimulus incited a sharp and premature inspiratory movement.

of the full series of shocks developed. In much the same way, a limited number of shocks, applied in mid expiration, helped the centre to attain the active state sooner, whereas a long series of shocks delayed its development.

influence of such stimulation is made to discharge sooner than it otherwise would and breathing is accelerated.

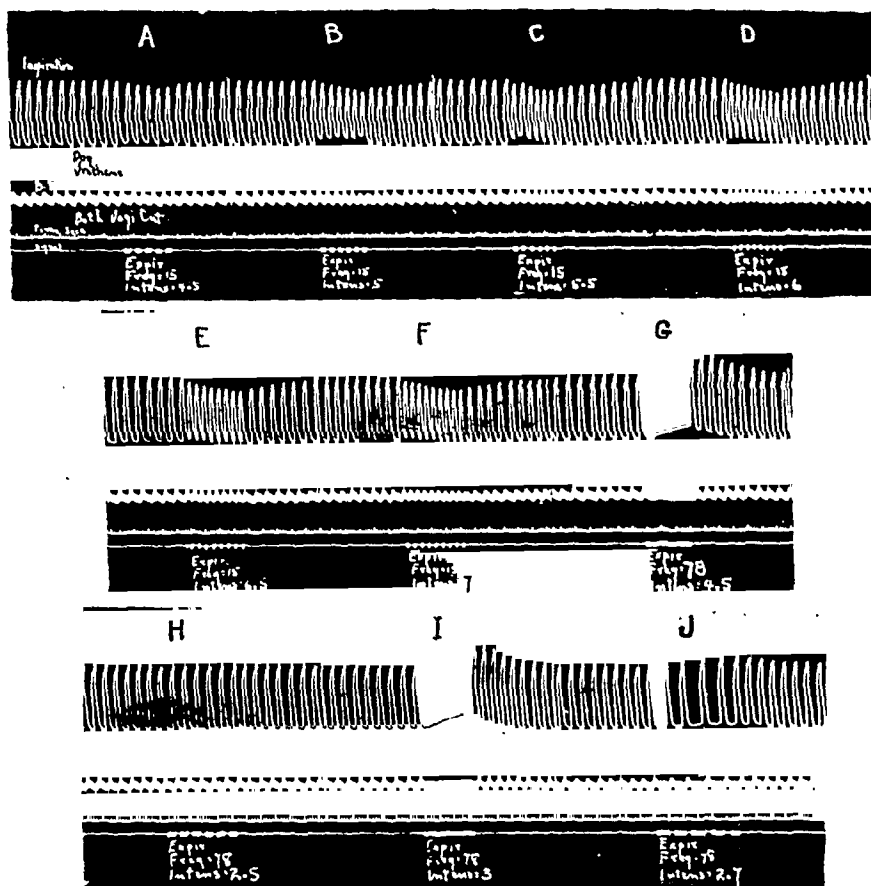


Fig. 3. Dog. Urethane. Expiratory stimulation A, B, C, D, E, and F, frequency of stimulus, 15. Intensity dial settings: 4.5; 5.0; 5.5; 6.0; 6.5; 7.0 respectively. More marked acceleration occurred with increasing intensity.

G, H, I, J, frequency of stimulus 78.

Intensity dial settings 4.5; 2.5; 3.0; 2.7 respectively. More marked slowing occurred with increasing intensity.

Note: Accurate assessment of the stimulus voltage *at the nerve* is difficult with electrodes of the liquid type used. The intensity of the stimulus cannot be stated in absolute values of voltage or current. But since the interelectrode resistance was high (<100,000 ohms) relative to the resistance of the linear voltage divider across the output of the stimulator (intensity control 10,000 ohms) the current drawn through the electrode systems was small. Intensity dial settings should therefore represent a linear voltage relationship.

Intensity dial settings were selected on the basis of observed physiological responses to stimulation.

High frequency expiratory vagal stimulation on the other hand caused a delay in the onset of each succeeding respiratory cycle, and thus slowed the breathing, apparently owing to inhibition of the respiratory centre. Figure 3 illustrates that these effects are due to the frequency rather than the intensity of the stimulus.

Increasing the intensity augmented the effectiveness of a stimulus but did not alter the type of response.

c. *Afferent vagal stimulation during the inspiratory phase only.* (*Inspiratory vagal stimulation.*) Table 3 shows that vagal stimuli which are effective in slowing the respiration when applied during expiration cause a marked respiratory

TABLE 2

*Effect on respiratory rate of progressive increase of stimulus frequency with constant intensity. Continuous stimulus. Dog. Urethane anesthesia*

STIMULUS FREQUENCY	RESP. RATE PRIOR TO STIMULUS	RESP. RATE DURING STIMULUS	% CHANGE IN RATE
15	28.2	34.8	+23.6%
22	31.2	39.6	+27.0%
30	29.4	40.8	+39.0%
43	34.8	45.0	+29.0%
78	36.0	22.8	-36.6%
92	25.0	8.0	-68.0%
130	22.8	0	-100.0%

TABLE 3

*Typical effects of vagal stimulation during inspiratory and expiratory phases. Urethane anesthetic*

EXPERIMENT	STIMULUS FREQUENCY	CHANGE IN RATE WITH EXPIRATORY STIMULUS	CHANGE IN RATE WITH INSPIRATORY STIMULUS
Rabbit.....	20	+17%	+3%
	162	-51%	+47%
Dog 1.....	20	+27%	+40%
	90	-69%	+142%
Dog 2.....	20	+29%	
	90	Arrest	+91%
Dog 27.....	18	+7%	
	78	-27%	+100%
Dog 29.....	18	+17%	+4%
	78	-25%	+70%
	108	-58%	

acceleration when applied during inspiration. Each inspiratory phase is reduced in both duration and depth and the subsequent expiratory phase is also shortened, a marked acceleration being the net result. As table 3 shows, even low frequency shocks are effective in producing respiratory acceleration when applied during the inspiratory phase. But the effect is slight compared with high frequency shocks from the point of view of both the acceleration and the reduction of tidal volume.

*Effect of Vagal Stimulation with Barbitone Anesthesia.* Experiments were performed on 8 dogs under soluble barbitone anesthesia (tables 4 and 5). Six of these dogs failed to show respiratory acceleration with low frequency stimuli (fig 4A and B). Two dogs did show an accelerator response and it is interesting to note that these results were obtained in animals which were lightly anesthetized, the usual dose of anesthetic having failed to produce complete anesthesia. In one of these animals (no. 39) increasing the dose of barbitone converted an accelerator response with low frequency stimulation to an inhibitory

TABLE 4  
*Effect of continuous vagal stimulation. Barbitone anesthesia*

EXPERIMENT	STIMULUS FREQUENCY	RESULTING CHANGE IN RESPIRATORY RATE
37	6.5 40	-64% Arrest
38	3.3	Arrest
39	10 41	+56% Arrest
41	10	Arrest
42	3.3 5.5 10	-41% -71% Arrest
43	3.3 5.5 10 20 41	+78% +66% +54% -12% Arrest
44	6 30 78	-25% -36% Arrest
45	3.4 22	-20% Arrest

response. Six of these animals however showed a marked respiratory acceleration with inspiratory stimuli (table 5) (fig.4B). One (no. 38) showed slowing even with this type of shock, indicating an inhibitory effect from each stimulus of sufficient degree and duration not only to cut short the inspiratory movement during which it was applied, but to delay the onset of the next inspiration.

*Effect of Limiting the Number of Shocks at an Inhibitory (High) Frequency.* In order to determine whether the respiratory responses to high and low rates of vagal stimulation depended upon the actual frequency of the stimulus, a series of experiments under urethane anesthesia was performed in which in each experiment

a particular frequency was employed, but the number of shocks at that frequency was varied. Having found a frequency and intensity of stimulus which caused

TABLE 5

*Effect of vagal stimulation during inspiratory and expiratory phases. Barbitone anesthesia*

EXPERIMENT	STIMULUS FREQUENCY	CHANGE IN RATE WITH EXPIRATORY STIMULUS		CHANGE IN RATE WITH INSPIRATORY STIMULUS	
		Per minute	%	Per minute	%
37	3.3	Arrest	-100		
	20	Arrest	-100		
	41	Arrest	-100		
	60	Arrest	-100		
	90	Arrest	-100	24 to 52	+113
38	3.3	Arrest	-100		
	90	Arrest	-100	52 to 44	-15
39	3.3	14.5 to 18.5	+28		
	10	15 to 16	+6.2	16 to 18.5	+15
	20	17 to 12	-14		
	41	19 to arrest	-100	16 to 20	+25
	90			14 to 25	+79
	With deeper anesthesia				
	3.3	15 to 16	+6.6		
	10	14 to 6	-57		
	20	16 to 4	-75		
	With still deeper anesthesia				
	3.3	12 to 4	-67		
	10	12 to arrest	-100		
	90			12 to 20	+67
40	3.3	12 to 6	-50		
	10	9 to 2	-78		
	90	15 to arrest	-100	13 to 17	+31
41	3.3	6.5 to 2	-69		
	10	6.5 to arrest	-100		
	90			8 to 16	+100
42	3.3	21 to 12	-43		
	5.5	18 to 3	-84		
	10	18 to arrest	-100	17.5 to 19.5	+11
	90			18 to 20	+11
43	5.5	21 to 30	+43		
	10	19 to 24	+26		
	20	19 to 18.5	-2.6		
	41	19 to 6	-68	13 to 22.5	+73

marked respiratory inhibition when applied throughout the expiratory phase, the same frequency and intensity was subsequently used, but the duration of stimu-

lation was limited to a short fraction of the expiratory phase, and the number of shocks thereby restricted. In this manner 10 or 20 shocks, for example, might be applied in mid-expiration at a frequency of 90 per second. Usually the stimulus switch on the arm of the respiratory recorder was adjusted so that the shocks were applied as the respiratory recording lever approached the resting level,

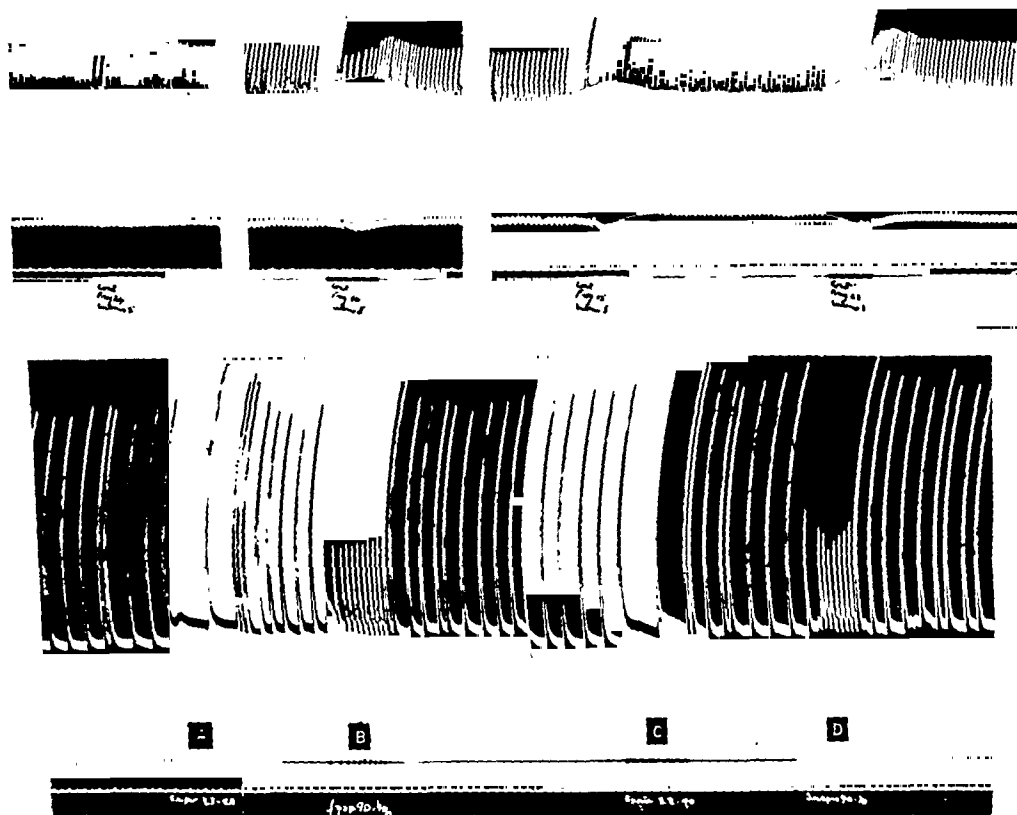


Fig. 4 A. (Upper) Dog. Soluble barbitone anesthesia. Continuous stimulus at constant intensity.

Frequencies of 3.4, 10, 15, and 22 cause respiratory slowing.

Fig. 4 B. (Lower) Dog. Soluble barbitone anesthesia. Expiratory stimulation at frequency of 3.3 per second causes respiratory slowing. (At A and C) Inspiratory stimulation with inhibitory stimulus, frequency 90, causes acceleration. (At B and D)

i.e., roughly in the middle of the expiratory phase. In some instances the switch was adjusted or worked manually so as to apply the shocks early or late in the expiratory phase.

Representative data are shown in table 6. As would be expected, in view of results from stimulation throughout the expiratory phase, if the duration of the stimulus was long enough (i.e., the number of shocks great) respiratory slowing invariably occurred. But when the number of shocks was restricted to a low

enough figure, even though the frequency was not changed; respiratory acceleration occurred. This acceleration was less marked than that resulting from low frequency stimulation, but it was nevertheless definite (fig. 5). From records

TABLE 6

*Effect of limiting the number of shocks. Central vagus. High frequency stimuli in expiration*

EXPERIMENT	STIMULUS FREQUENCY	NUMBER OF SHOCKS	NORMAL RESP. RATE PER MIN.	RESP. RATE WITH STIMULATION	PER CENT CHANGE IN RESP. RATE
3	90	2	17.4	21.6	+24%
		5	16.8	17.4	+3.6%
		10	16.8	14.1	-16%
		20	16.8	12.6	-25%
4	90	10	14.4	15.6	+8.3%
		40	15	13.2	-12%
6	60	4	22.8	24.0	+5.3%
		12	22.2	20.4	-8.1
15	78	1	23.2	22.4	-3.5%
		5	25.2	23.6	-6.3%
17	78	7	23	27.2	+18%
		12	23.2	24	+3.4%
		19	24	20.5	-14.5%
		36	24	17.6	-27%
19	78	8	24.6	29.0	+18%
		38	23.8	23.0	-3.4%
20	130	8	26	33.6	+29%
		37	26.4	24	-9.1%
		46	26.8	22.4	-16%
		98	26.4	18.6	-30%
23	90	20	10.5	12.3	+17%
		40	10.5	9.9	-5.7%
46	78	14	19.8	21.6	+9%
		43	19.8	18.6	-6%
47	130	5	28.8	31.6	+9.7%
		23	28	24.8	-11%
48	90	40*	11.3	13.3	+18%

\* This was the largest number of shocks at a high frequency that caused acceleration.

on rapidly moving paper this acceleration was shown to be due to a reduction in the duration of the expiratory phase, resulting in a definite reduction in the duration of each complete respiratory cycle (from inspiration to inspiration) (fig. 6.

table 7). There were times when no acceleration occurred even with as few as 3 or 4 shocks or even a single shock (expt. 15—table 6) and such instances were frequently associated with a poor accelerator response to low frequency stimuli. In almost all instances a number of shocks could be determined intermediate between those causing acceleration and slowing which resulted in no change in respiratory rate.

TABLE 7

*Effect of brief volleys at rapid frequency on the duration of the phases of the respiratory cycle.  
Expiratory stimuli*

STIMULUS FREQUENCY	NUMBER OF SHOCKS	DURATION OF INSPIR.		DURATION OF EXPIR.		DURATION TOTAL CYCLE	
		Normal	With stim.	Normal	With stim.	Normal	With stim.
		seconds	seconds	seconds	seconds	seconds	seconds
90	40*	3.0	3.0	2.3	1.5	5.3	4.5
90	5	2.0	2.0	1.6	1.0	3.6	3.0
78	27	1.2	1.2	1.9	1.5	3.1	2.7
78	2	2.2	2.2	1.6	1.3	3.8	3.5

\* See footnote to table 6.

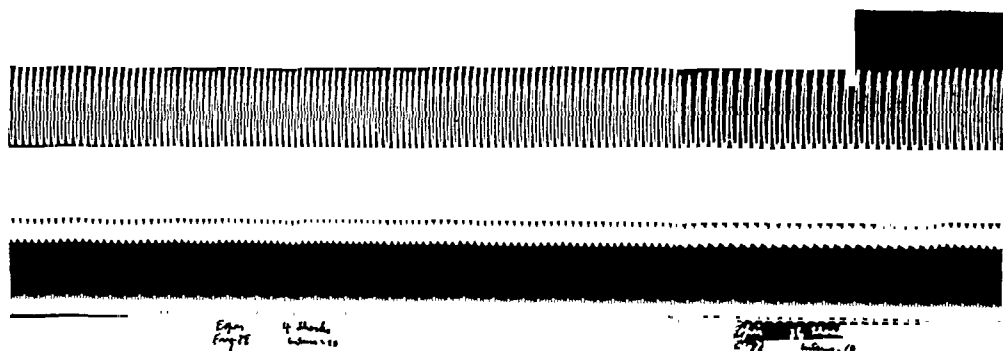


Fig. 5. Dog. Urethane.

Left. 4 shocks applied in mid-expiration at a frequency of 78 per second cause acceleration of respiration. Normal respiratory rate 24 per min. Rate during stimulus, 28 per min.

Right. 36 shocks applied in mid-expiration at frequency of 78 per second cause respiratory slowing. Normal respiratory rate 24 per minute. Rate during stimulus, 17.4 per minute.

Some further observations pertinent to the discussion to follow were also made. If a stimulus was employed which was of high frequency and long duration, and which would thus delay the onset of the next inspiration if applied in mid expiration, this same stimulus sometimes failed to delay the inspiration about to occur if it were applied very late in the expiratory phase, i.e., a fraction of a second before the expected inspiratory movement. When so timed by manual use of the switch, it frequently precipitated a sharp and slightly premature inspiration

(fig. 7). The explanation for this phenomenon appears to be that the first few impulses to reach the centre threw it into activity before the inhibitory influence

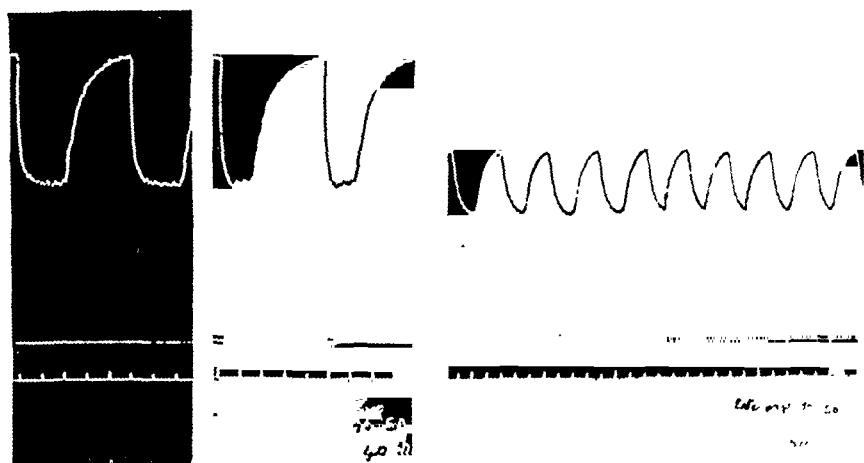


Fig. 6. Dogs. Urethane. Rapid kymograph records showing that acceleration resulting from a limited number of shocks in expiration is due to the inciting of an earlier inspiration, with reduced duration of expiratory phase. Normal curves at left; effect of stimulus (indicated above time lines) shown at right.

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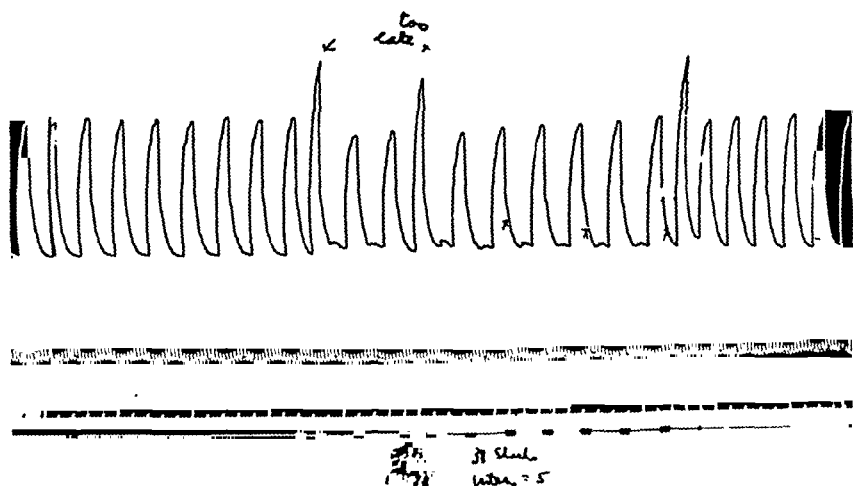


Fig. 7. Dog. Urethane. Stimulus switch worked manually; 38 shocks at frequency of 78 applied in mid-expiration usually delayed the next inspiration. When applied too late in expiratory phase the same stimulus incited a sharp and premature inspiratory movement.

of the full series of shocks developed. In much the same way, a limited number of shocks, applied in mid expiration, helped the centre to attain the active state sooner, whereas a long series of shocks delayed its development.

By contrast, if a brief series was sent in at the beginning of expiration, (i.e., when the lever just started to fall from the peak of inspiration) its accelerating influence was largely lost. Apparently little augmentor effect resulted from the afferent impulses when they impinged upon cells whose irritability was low as a result of their activity a moment previously.

*Tonus.* One other feature of the results obtained on dogs under urethane was that the tonus or resting expiratory level was also changed in a manner related to the frequency of the stimulus in most cases. A low frequency generally though not invariably caused a rise of tonus, i.e., a greater resting lung volume; and a high frequency generally caused a lower tonus or a smaller resting lung volume (fig. 1B). These results are also in agreement with those previously observed in rabbits with the exception that it was occasionally found in rabbits that the increase in tonus was so marked that the pointer reached and maintained a level corresponding to a full inspiration (see also 26). Tonus changes with barbitone tended to be slight and sometimes none occurred with any frequency of stimulus. At times they were paradoxical (fig. 4A).

*DISCUSSION.* A résumé of the above statements can be presented briefly. Under urethane, stimulation of the central end of the vagus nerve with a low frequency even when limited to the expiratory phase of each respiratory cycle produced a respiratory acceleration associated with a rise of tonus and decreased tidal volume. The acceleration was due primarily to a shortening of each expiratory phase resulting in the earlier onset of each inspiration. High frequency stimulation under similar conditions on the other hand slowed the respiration by prolonging each expiratory phase and delaying the onset of each inspiration. This effect was associated with a fall in the respiratory tonus. If the duration of the high frequency expiratory stimulus was restricted so that only a limited number of shocks was sent in, a respiratory acceleration occurred, which was due to a shortening of the expiratory pause and the instigation of an earlier inspiration. As the number of shocks increased a value was reached which failed to alter the respiratory rate. A number of shocks greater than this led to respiratory slowing which became more marked with further prolongation of the stimulus. Under barbitone acceleration resulted with low frequency stimulation in only two out of eight dogs and these were both lightly anesthetized. Respiratory acceleration was also produced by central vagal stimulation at a high frequency when it was restricted to the inspiratory phase. The response under these conditions was largely due to the shortening of each inspiratory phase though some reduction of the expiratory phases also occurred. This type of activity was studied in detail by Adrian (1), and by Boyd and Maaske (5) and appears to be analogous to the stretch reflexes from the lungs. It was demonstrated to be present under urethane and barbitone. It is a response resulting from the appropriate inhibition of the inspiratory centre during its active phase. In this respect it differs from the acceleration resulting from low frequency stimulation under urethane, which precipitates the onset of inspiratory activity, even when restricted to the expiratory phase. Two different types of respiratory acceleration thus appear to be demonstrable.

Much evidence, sometimes controversial, has been produced by various workers

interpreted to indicate that certain augmentor and inhibitory respiratory reflexes mediated by the vagi depend on activity of different sets of fibres in those nerves. The experiments of Adrian (1), Hammouda et al. (13, 14), and Knowlton and Larrabee (20, 21) suggest that there are different types of fibres of pulmonary origin in the vagi which may produce various respiratory reflexes. The experiments of Partridge (22) and Comroe (9) indicate that some of the vagal fibres are of cardio aortic origin (also 18). It seems highly probable therefore that various respiratory responses resulting from vagal stimulation would sometimes be due to activation of specific types of fibres with particular forms of stimulation. However, certain arguments weigh against this explanation of the different respiratory responses obtained in these experiments. In the discussion to follow technical factors are of critical importance in the interpretation of the results.

In the first place, with the stimulator used the wave form of each single shock potential (a series of which constituted the stimulus) was constant no matter what frequency was used. It seems probable therefore that the type of fibre being stimulated would not vary with the stimulus frequency. It is to be expected that if each single discharge from the stimulator (having a fixed form and potential for a given experiment) was capable of selecting a particular type of fibre it would do so whether the shocks were repeated at long or short intervals. The possibility is admitted that at the more rapid frequencies the summation of subminimal stimuli might bring into play fibres of higher threshold which did not respond to the low frequency shocks, if a submaximal intensity was used. In this case increasing the intensity without changing the frequency should arouse activity in these less irritable fibres and convert an accelerator to an inhibitory response. Such reversal did not occur upon increasing the intensity, the effects remaining qualitatively the same, and characteristic of the frequency used. Further, the fact that the type of anesthesia altered the response to vagal stimulation suggests that the characteristic influence of low and high frequency shocks is dependent upon a central action. The effect of the anesthetic upon the nerve fibres being stimulated was probably slight under the conditions of these experiments since the nerve lay in a liquid type of electrode removed from the active circulation, so that any anesthetic would be washed out of it. The last and perhaps most important evidence lies in the results obtained with a limited number of shocks at high frequency in expiration. Under these conditions not only the wave form and intensity, but also the frequency of the stimulus were constant for a particular experiment. The apparent effect of the afferent discharge frequency in controlling the respiratory response must therefore be explained by some qualitative or quantitative concept that allows not only for the gross frequency effects, but for the opposite effects of a small and a large number of shocks at a given frequency. If these arguments are correct it must be assumed that both low and high frequency stimuli activate the same set of fibres in the nerve, and that different respiratory responses to them must be the result of a selective action of the respiratory centre or its neuronal connections with the afferent pathway.

There is other evidence that brain centres may act selectively to different stimulus frequencies. Hare and Geohegan (15) and Berry, McKinley and Hodes (3) have shown that by direct stimulation of the hypothalamus blood pressure changes may be caused, the directions of which are dependent upon the frequency of the stimulus. Ashkenaz (2) has also demonstrated that pressor or depressor responses can be elicited upon stimulation of the sciatic, tibial and ulnar nerves by controlling the frequency of the stimulus, though in this case mediation by different fibre groups cannot be ruled out.

Gesell and Moyer (11) have presented an explanation for the augmentor effects of vagal pulmonary afferent paths, based upon the reciprocal inhibition of one division of the respiratory centre by the other half centre during activity of the latter. But the dependence of different respiratory responses upon the *manner* i.e., the frequency at which, or the time during which a given pathway works, implies a selective action within the central nervous system of another type. The nature of the selective action at the central cells can only be surmised. It is suggested that each vagal impulse reaching the brain may produce a specific state. The reception at the centre of a sequence of such impulses might determine an increase in the degree or extent of this state depending on the rate at which the impulses were received. Assuming some restoration process to go on as soon as the central state developed it might be expected that a low frequency stimulus or a limited number of shocks at high frequency would result in a build-up to a lesser degree than would occur with a prolonged high frequency stimulus. It is further suggested that the effect of this state, whether augmentor or inhibitory, depends on its concentration. Such action may be compared with the effects of certain drugs. Nicotine, for example, is known to stimulate autonomic ganglia in low concentrations and to paralyse them in higher concentrations. Dale and Laidlaw (10) cite an interesting observation relative to this argument. Prolonged stimulation of the chorda tympani resulted in a biphasic secretory response. When the stimulation was maintained the rate of secretion, at first rapid, fell off but increased again for a time after the stimulus was stopped. It has been shown by Brown and Feldberg (8) that acetylcholine promotes activity of perfused autonomic ganglia in low, and depresses their activity in high concentrations. The presence of excessive acetylcholine also depresses the contraction of striated muscle, though this substance stimulates contraction in weaker concentration (6, 7). These observations offer support in a broader sense for the idea that the respiratory responses to vagal stimulation, viz., acceleration or slowing, may depend upon the degree or extent of a central state resulting from afferent impulses received by the central mechanism (both effects being mediated by the same sensory fibres), rather than upon central states of different type (excitatory and inhibitory) built up by the activity of different sets of fibres. This concept will subsequently be referred to as the concentration hypothesis.

A number of respiratory phenomena can be interpreted in the light of such a theory. For example, the lung stretch reflexes and inspiratory vagal stimulation cause the respiration to be more rapid than when these influences are absent.

It has been shown (these expts. 1, 24) that the more rapid rate is due primarily to the cutting short of the inspiratory phase, but partly also to a shortening of each expiratory phase. On the basis of the concentration hypothesis, the inhibition of inspirations can be explained by the rapid development of an inhibitory state by the high frequency discharge of the pulmonary fibres while the lungs are stretched. The reduced duration of the expiratory phases may be explained by the concept that, during the dissipation of the central state which had been of an inhibitory degree, a stage would be passed through when its concentration would be slight enough to be augmentor in effect, thus inciting the earlier onset of the subsequent inspiration.

It might also be expected that under conditions when inhibitory processes are very marked, the inhibition created by high frequency vagal stimulation would last longer than normally. Evidence for this was seen in one of the dogs under barbitone. In this animal a prompt inhibition of inspiration occurred with high frequency inspiratory vagal-stimulation so that the tidal volume was much reduced and each inspiratory phase shortened. There was nevertheless a marked prolongation of the expiratory phases to such a degree in fact that the respiratory rate was reduced in spite of the shortened inspiratory phases. Further, in instances where the frequency of a stimulus was close to the inhibitory range it might be expected that after repetition and as a result of a cumulative action, the response would pass over from an accelerator to an inhibitory one. This has been observed (fig. 1B).

Because of the influence of afferent vagal impulse frequency on respiratory responses, experiments which might modify, but not eliminate, vagal conduction (e.g., partial freezing and narcotization) must be critically appraised. In the light of experiments by Tasaki (24) and Blair and Erlanger (4) it is conceivable that a reduced impulse frequency may occur beyond a region of partial block. Such a reduction in the afferent impulses might lead to responses not to be expected from a knowledge of the impulse frequency in front of the block only.

It is interesting that rapid vagal stimulation, especially when applied late in the expiratory phase, frequently precipitates a sudden and deep inspiratory movement, when it might be expected to prolong the expiratory pause (fig. 7). This may be explained by the concentration theory as a manifestation of the excitatory effect of the first few impulses, precipitating the centre (almost ready to discharge spontaneously) into action before the inhibitory degree of the central state could develop. Such sudden deep inspirations differ from those seen in figure 4A, where  $\text{CO}_2$  accumulation occurred during the prolonged expiration, and presumably caused the ultimate break through the inhibitory stimulus.

It must be pointed out that certain observations in these experiments are more difficult to interpret in the light of the concentration theory. For example, under barbiturate anesthesia acceleration with low frequency expiratory stimuli characteristically failed to occur. The expiratory phases were usually not shortened, but rather prolonged, and the breathing rate decreased. Yet inspiratory stimuli which cut short inspiration promptly also caused a shortening of the subsequent expiratory phase in most experiments. That is, in terms of

the concentration theory, following the inspiratory inhibition the concentration of the central state progressively decreased and passed through a phase which was augmentor in effect, thus promoting the next inspiration and shortening the expiratory phase. Yet it was usually not possible to duplicate the latter effect by low frequency expiratory shocks under barbitone. Even such infrequent impulses appear to have built up the central state to an inhibitor degree. It is worthy of note however, that at times a limited number of expiratory shocks (usually very small) did cause acceleration. Our experience with the barbiturates suggests that inhibitory processes are marked. It is conceivable therefore that the augmentor level of the central state is too critical to be created with reliability by artificial stimulation without exceeding that level and establishing an inhibitory effect. It would on the other hand be produced as a transient state during the dissipation of an inhibitory central state, as with inspiratory inhibitory stimulation.

If it is of broader application than can be shown in the limited scope of these experiments, and if it can be proven valid by further experimentation the concept that the pattern of reflex responses may depend not only upon the type of afferent fibre and pathway involved, but also upon the rate at which the afferent pathways are activated has important implications. It would add a further complexity to the overall picture of reflex action, and provide another mechanism by which the responses evoked by sensory stimulation may be modified.

#### SUMMARY

1. Under urethane anesthesia low frequency central vagal stimulation in dogs caused respiratory acceleration. High frequency stimulation continuously or during expiration slowed the breathing by prolonging the expiratory phase, unless the duration of the stimulus was limited so that only a small number of shocks was applied, when respiratory acceleration occurred. Acceleration was also caused by high frequency afferent vagal stimulation during inspiration, i.e., by intermittent inspiratory inhibition analogous to the lung stretch reflexes.

2. Under barbiturate anesthesia respiratory acceleration with low frequencies of vagal stimulation usually failed to occur, and these stimuli usually inhibited the breathing. Vagal stimulation during the inspiratory phase alone did cause respiratory acceleration in most instances even under barbitone.

3. In explanation of the results, a theory was presented in which it was suggested that each afferent vagal impulse serves as a unitary contributor to the development of a central state affecting the respiratory centre or its connections. Under urethane a low frequency stimulus or a limited number of impulses at high frequency was considered to result only in a slight build-up of this central state. A rapid and sufficiently prolonged stimulus was considered to result in development of the central state to a greater degree. Comparisons were made with the actions of various drugs in weak and strong concentrations. It was suggested that when the central state was of slight extent or degree, its effect on the respiratory centre was augmentor; when of greater extent or degree, its effect was inhibitor. With barbitone the failure of respiratory acceleration to occur

with low frequency stimuli was attributed to the effect of this drug in accentuating the development of an inhibitory level of the central state.

4. The results of the experiments reported upon were interpreted to indicate that whether acceleration or inhibition resulted from different forms of vagal stimulation the type of response was due to a central selective action to impulses conveyed in a single type of afferent path, rather than to stimulation of specific augmentor and inhibitor types of fibres in the vagi.

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# THE RESPIRATORY AND CIRCULATORY RESPONSE OF NORMAL MAN TO INHALATION OF 7.6 AND 10.4 PER CENT CO<sub>2</sub> WITH A COMPARISON OF THE MAXIMAL VENTILATION PRODUCED BY SEVERE MUSCULAR EXERCISE, INHALATION OF CO<sub>2</sub> AND MAXIMAL VOLUNTARY HYPERVENTILATION<sup>1</sup>

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The effects of inhalation of low concentrations of CO<sub>2</sub> have been investigated widely in man (1, 2, 3, 4). The effects produced by higher concentrations (7.5–10 per cent CO<sub>2</sub>) have been recorded incompletely and only upon small groups. To supplement our knowledge of the quantitative aspects of respiration and circulation it seemed worthwhile to measure the response of a large group of normal subjects to these higher concentrations of carbon dioxide. In addition the maximal respiratory minute volume produced by inhalation of 7.6 per cent and 10.4 per cent CO<sub>2</sub> was compared with that resulting from exhausting muscular exercise and maximal voluntary hyperventilation.

**METHODS.** Forty-four male medical students between the ages of 21 and 26 served as subjects. Eighteen of these had just completed a six weeks program of athletic training and were in excellent physical condition.

A typical experiment was conducted as follows: The subject sat either upon a chair or a stationary bicycle. No attempt was made to secure a basal state. An aviation type half-mask was strapped tightly to the face. The subject breathed through rubber valves (Japanese gas mask type) which offered minimal resistance. Expired air was conducted through tubing 1½ inches in diameter to a balanced compensating 300 liter spirometer. Measurement of the volume of expired air (not corrected to S.T.P.) respiratory rate, pulse rate and systolic and diastolic blood pressure were recorded. Some or all of the following procedures were then completed: 1, determination of maximal voluntary breathing capacity (5) over a half-minute period; 2, inhalation of "7.6 per cent CO<sub>2</sub>" in oxygen (mixtures varied from 7.4 to 7.8 per cent CO<sub>2</sub>); 3, inhalation of 10.4 per cent CO<sub>2</sub> in oxygen, and 4, exercise at a maximal rate upon a heavily loaded stationary bicycle. This exercise consisted of a "warm-up" period of several minutes on the bicycle followed by exertion at a maximal rate of pedalling for 60–75 seconds. All subjects except one considered this to be exhausting. Carbon dioxide mixtures were supplied in 6,000 liter high pressure tanks. Each tank was connected through a regulator to a 10 liter rubber bag which was attached to the

<sup>1</sup>This work was performed under contract with the Medical Division, Chemical Warfare Service, Edgewood Arsenal, Maryland.

inspiratory valve of the mask when desired. The bag was kept approximately half full during periods of  $\text{CO}_2$  inhalation. The system did not permit re-breathing. The inhalations of  $\text{CO}_2$  were continued until the respiratory minute volume reached a plateau (not more than 10 per cent variation during four consecutive thirty second periods) or until the subject became definitely uncomfortable. After each procedure, the subject was allowed to have a 5-10 minute rest. During this period, the subject breathed room air. Twenty-three subjects performed the maximal voluntary breathing capacity test, 42 inhaled 7.6 per cent  $\text{CO}_2$ , 31 breathed 10.4 per cent  $\text{CO}_2$  and 25 exercised on the bicycle.

RESULTS. 1. *Respiration*. The maximal minute volume, rate and depth of breathing attained by the subjects inhaling 7.6 and/or 10.4 per cent  $\text{CO}_2$  are recorded in table 1. The averages of the maximal minute volumes were 51.5 liters per minute for 7.6 per cent  $\text{CO}_2$  and 76.3 liters per minute for 10.4 per cent  $\text{CO}_2$ . Figure 1 shows graphically the average maximal minute volume of subjects breathing 7.6 and 10.4 per cent  $\text{CO}_2$ . For sake of completeness it includes the data of Shock and Soley (1, 2) and of Heller, Killiches and Drinker (4) dealing with the respiratory responses to 1, 2, 4 and 5 per cent  $\text{CO}_2$  inhalation. The extremes encountered in our experiments were widely separated: 24 to 102 liters/min. for 7.6 per cent  $\text{CO}_2$  and 40 to 130 liters/min. for 10.4 per cent  $\text{CO}_2$ . These marked variations could not be correlated with height, weight, surface area, age or maximal breathing capacity.

Plateaus for minute volume were reached in only 27 of 42 individuals breathing 7.6 per cent  $\text{CO}_2$  at times varying from 2.5 to 8.5 minutes (average 5.8 min.) and in 13 of 31 subjects inhaling 10.4 per cent  $\text{CO}_2$  at times ranging from 2.5 to 6.0 minutes (average 3.5 min.).

The increase in respiratory minute volume was achieved largely by an increase in depth of breathing. Depth of breathing increased approximately four fold while the rate was little more than doubled. The failure of rate to increase until high concentrations of  $\text{CO}_2$  are inhaled has been noted by Haldane (3).

When the  $\text{CO}_2$ - $\text{O}_2$  mixtures were discontinued, respiration returned rapidly to normal. One minute after discontinuing 7.6 per cent  $\text{CO}_2$ , average respiratory minute volume was 200 per cent of normal, at 2 minutes it was 73 per cent above normal and at 3 minutes it was 29 per cent above normal. One minute after discontinuing the inhalation of 10.4 per cent  $\text{CO}_2$ , the average respiratory minute volume was 220 per cent above normal, at 2 minutes, 38 per cent and at 3 minutes respiratory minute volume had returned to normal values.

Twenty-seven subjects breathed both 7.6 and 10.4 per cent  $\text{CO}_2$  mixtures. In 25 of these, inhalation of the higher concentration led to further increase in minute volume of respiration but in 2 the maximal minute volume of respiration was slightly lower when 10.4 per cent was breathed. In one of these steady maximal values had been reached on both occasions and it appears that 10.4 per cent  $\text{CO}_2$  was above the optimal concentration of  $\text{CO}_2$  required to produce maximal respiratory stimulation in this subject. In the other maximal values were not reached on either occasion and consequently a similar conclusion is not warranted.

A comparison was made in 19 subjects of the maximal ventilation produced

TABLE 1

The maximal effect of 7.6 and 10.4 per cent CO<sub>2</sub> upon rate, depth and minute volume of respiration

	7.6% CO <sub>2</sub> (42 SUBJECTS)		
	Rate/min.	Depth (lit.)	Min. Vol. L/min.
Average.....	28.4	2.1	51.5
Range.....	16-72	0.86-3.12	24-102
Standard dev.....	10.8	0.56	18.4
	10.4% CO <sub>2</sub> (31 SUBJECTS)		
	Rate/min.	Depth (lit.)	Min. Vol. L/min.
Average.....	34.9	2.45	76.3
Range.....	20-89	1.40-3.66	40-130
Standard dev.....	15.1	0.49	24.8

TABLE 2

Comparison of maximal respiratory minute volumes produced by inhalation of 7.6 and 10.4 per cent CO<sub>2</sub>, severe muscular exercise and maximal hyperventilation in 19 normal men

	MAX. BREATHING CAPACITY	7.6% CO <sub>2</sub>	10.4% CO <sub>2</sub>	MUSCULAR EXERCISE
Average.....	166	48.9	71.4	109.6
Range.....	132-198	26-86	40-114	80-140
Standard dev...	20.3	15.4	21.4	18.3
% of M.B.C....	100	29	43	66

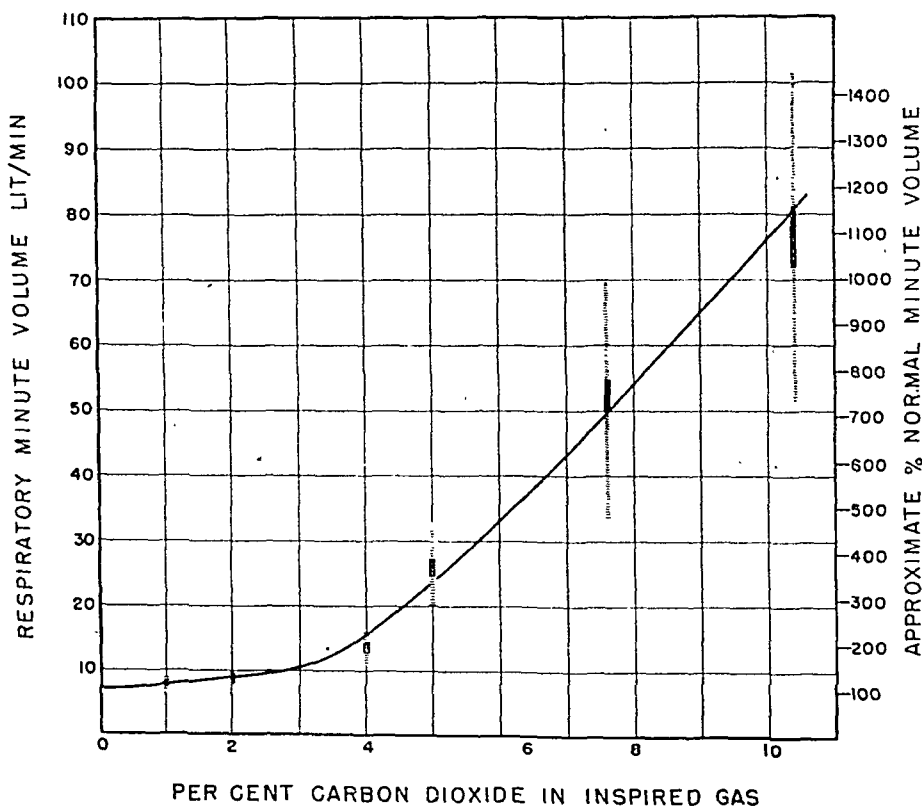


Fig. 1. Respiratory response to CO<sub>2</sub>. The data of Shock and Soley for 1, 2 and 4 per cent CO<sub>2</sub> in air and those of Heller et al., for 5 per cent CO<sub>2</sub> in air are included. The hatched line represents one standard deviation on each side of the mean. The solid bar represents the standard error of the mean on each side of the mean. The smoothest possible curve is drawn through the solid bars.

a, by inhalation of 7.6 and 10.4 per cent CO<sub>2</sub>; b, by severe muscular exercise, and c, by voluntary hyperventilation (maximal breathing capacity) (table 2). The averages were 48.9 liters for 7.6 per cent CO<sub>2</sub>, 71.4 for 10.4 per cent CO<sub>2</sub> inhalation, 109.6 for muscular exercise and 166 liters per minute for voluntary hyperventilation. Our values for maximal breathing capacity are higher than those reported by others (5); this is probably due to the fact that our subjects breathed through both mouth and nose instead of through a mouthpiece alone. Our figures for the maximal ventilation during severe exercise are as high or higher than those previously reported; three of our subjects had minute volumes in excess of 130 liters per minute. In only seven instances was the hyperpnea produced by CO<sub>2</sub> as much as 50 per cent of the maximal breathing capacity and in only two cases was it more than 60 per cent (65 and 71 per cent) of the maximal breathing capacity. In only two subjects did the hyperpnea produced by CO<sub>2</sub> inhalation exceed that produced by severe muscular exercise; in one of these the load on the bicycle was insufficient to cause exhausting work.

TABLE 3  
*Maximal circulatory effects produced by CO<sub>2</sub> inhalation*

	NUMBER OF SUBJECTS	CHANGE IN PULSE RATE PER MIN.		NUMBER OF SUBJECTS	INCREASE IN SYSTOLIC B. P. MM. HG		NUMBER OF SUBJECTS	INCREASE IN DIASTOLIC B. P. MM. HG	
		Average	Range		Average	Range		Average	Range
7.6% CO <sub>2</sub>	42	+16.7	-10 to +68	34	30.8	12 to 64	34	22.2	4 to 47
10.4% CO <sub>2</sub>	29	+15.6	-4 to +36	29	33.4	10 to 52	29	25.0	3 to 62

2. *Circulation.* The average of the maximal observed increases in pulse rate, systolic blood pressure and diastolic blood pressure produced in each subject by inhalation of 7.6 and 10.4 per cent CO<sub>2</sub> are shown in table 3. The circulatory changes from subject to subject were just as variable as the respiratory responses though there was little correlation between the two; the several individuals who had the greatest increases in respiration with CO<sub>2</sub> inhalation did not have the greatest increases in circulation. The 7.6 per cent CO<sub>2</sub> mixture was breathed for an average of 7.4 minutes. In 30 of the 34 subjects blood pressure reached a plateau (fluctuations over a two minute period were less than 10 per cent). The 10.4 per cent CO<sub>2</sub> mixture was inhaled for an average of 3.8 minutes and blood pressure determinations during the inhalation became stabilized in only 13 of the 30 individuals. The data in table 3 suggest that 10.4 per cent CO<sub>2</sub> was no more potent a circulatory stimulant than the 7.6 per cent mixture. This interpretation may not be justified because of the relatively brief exposure to the higher concentration.

Immediately following the removal of the bag containing CO<sub>2</sub> mixtures, the diastolic blood pressure often fell abruptly, while the systolic pressure decreased only slightly. The average immediate fall in diastolic pressure in the 29 subjects

breathing 10.4 per cent CO<sub>2</sub> was 31.2 mm. Hg and in systolic pressure was only 7.4 mm. Hg. The fall in diastolic pressure was usually followed by an acceleration of the pulse and a prompt return toward previous figures. This phenomenon had been noted previously by Goldstein and DuBois (6).

3. *Symptoms incident to inhalation of CO<sub>2</sub>.* The symptoms most frequently noted during or following inhalation of 7.6 per cent CO<sub>2</sub> were headache in 23 (55 per cent), dizziness in 14 (33 per cent) and dyspnea in 13 (31 per cent). The symptoms noted most often incident to inhalation of 10.4 per cent CO<sub>2</sub> were dizziness in 18 (58 per cent), headache in 13 (42 per cent) and dyspnea in 10 (32 per cent) (table 4). Seventy-two per cent of the headaches occurred either in the period immediately after withdrawal of the CO<sub>2</sub> mixture or became accentuated at that point. In three subjects inhalation of 10.4 per cent CO<sub>2</sub> abolished a headache which had been initiated by 7.6 per cent CO<sub>2</sub> and persisted after

TABLE 4  
*Major symptoms incident to CO<sub>2</sub> inhalation*

	7.6% CO <sub>2</sub> (42 SUBJECTS)		10.4% CO <sub>2</sub> (31 SUBJECTS)	
	During inhalation	Only after inhalation	During inhalation	Only after inhalation
Headache.....	1	12	3	10
Dizziness.....	9	5	13	5
Dyspnea.....	13	0	10	0
Sweating.....	8	1	6	1
Faintness.....	0	0	7	1
Restlessness.....	0	0	5	0
Fullness in head.....	3	1	3	0
Unconsciousness.....	1	0	3	0

the shift to room air. Forty-one per cent of the dizziness was first noted or accentuated in the immediate post inhalation period. These symptoms appeared at the same time as the abrupt fall in diastolic blood pressure. However, the average drop in diastolic blood pressure of those subjects with post CO<sub>2</sub> headache or dizziness was exactly the same as for the group which failed to note these symptoms.

Other symptoms noted were: Irritation of nose, palpitation, mental clouding, dimness of vision, muscle tremor or twitching, "generally uncomfortable", tingling, cold extremities, exhaustion, mental depression, substernal pain and "sensation as though in first stage of nitrous oxide anesthesia."

It is interesting to note the levels of ventilation at which dyspnea was noted. Those who noted no dyspnea had maximal minute volumes ranging from 24 to 114 (average 60 liters per min.), those who noted slight to moderate dyspnea had maximal minute volumes ranging from 29 to 110 (average 62.7 liters per min.) and those who had marked dyspnea had minute volumes between 50 and 130 liters per minute (average 86.8).

**DISCUSSION.** Marked individual variations in respiratory and circulatory responses to  $\text{CO}_2$  inhalation have been noted previously. Figure 1, which illustrates the average maximal minute volumes, with one standard deviation and a standard error of the mean on either side of the mean, shows that these individual differences are most pronounced when high concentrations of  $\text{CO}_2$  are inhaled. Low concentrations of  $\text{CO}_2$  act almost wholly on the medullary centers while high concentrations may act upon the chemoreceptors as well (7). Consequently this variability of response to high concentrations of  $\text{CO}_2$  can be due to differences in sensitivity of either the medullary center or of the chemoreceptors. It is known that human chemoreceptors vary widely in their response to another stimulus, anoxia (8). Six of our subjects who had excellent respiratory responses to 7.6 per cent  $\text{CO}_2$  inhalation were given 10 per cent  $\text{O}_2$  to breathe; in each case a poor response to anoxia was observed. This is only presumptive evidence however that the chemoreceptors were not involved in the  $\text{CO}_2$  response, since their sensitivity to low  $\text{O}_2$  and to high  $\text{CO}_2$  tension might not run parallel. Since the individual variations could not be correlated with height, weight, surface area, maximal breathing capacity or with chemoreceptor response to anoxia, it is probable that they represent differences in the sensitivity of the medullary centers to increased  $\text{CO}_2$  tension.

It is not certain that the maximal responses obtained in this study represent the maximal capacity of an individual to react to  $\text{CO}_2$  since we did not determine responses to concentrations of  $\text{CO}_2$  higher than 10.4 per cent. Brown (9) exposed 7 subjects to 12.4 per cent  $\text{CO}_2$  and found that the respiratory responses in six were less than when 10.4 per cent  $\text{CO}_2$  was inhaled. Since 12.4 per cent  $\text{CO}_2$  could be inhaled only two minutes, it is possible that sufficient time did not elapse for full  $\text{CO}_2$  action. On the other hand, it must be remembered that the observed effect of  $\text{CO}_2$  is the algebraic sum of two factors: 1, a direct stimulant effect of  $\text{CO}_2$  upon the medullary centers and chemoreceptors, and 2, a narcotic action (10) which tends to depress the respiratory center. It appears probable that despite the vigorous hyperpnea in our subjects a narcotic effect was occurring simultaneously. In support of this view is the finding that 33 per cent of those breathing 7.6 per cent  $\text{CO}_2$  complained of dizziness and 58 per cent of those inhaling 10.4 per cent  $\text{CO}_2$  had this symptom. Seven (23 per cent) of those breathing 10.4 per cent  $\text{CO}_2$  felt as though they were about to faint, 2 (6 per cent) were completely unaware of their surroundings, 2 (6 per cent) noted a similarity to the onset of nitrous oxide anesthesia, 1 (3 per cent) became unconscious and 1 (3 per cent) noted analgesia.

Two other aspects of this narcotic effect are of interest. First, it is probable that inhalation of high concentrations of  $\text{CO}_2$  in the treatment of individuals with depressed medullary centers (due to anesthesia, morphine or barbiturate poisoning, carbon monoxide poisoning) may produce further narcosis, whether hyperpnea or hypertension develops or not. Consequently, if increased ventilation alone is desired, it might be preferable to produce this by mechanical means. When other effects of  $\text{CO}_2$ , such as cerebral vasodilatation, shift in  $\text{HbO}_2$  or  $\text{HbCO}$  dissociation curves are indicated, the narcotic effect of  $\text{CO}_2$  must be borne

in mind. Second, the narcotic effect might be a partial explanation of the finding that inhalation of high concentrations of CO<sub>2</sub> produces only 43 per cent (average) of the maximal ventilation possible by voluntary hyperventilation. Since many physiologists believe that CO<sub>2</sub> is the most potent stimulant to respiration, its inhalation should produce higher minute volumes unless inhibitory factors act concurrently. One inhibitory factor may arise from the increase in blood pressure, which through pressure receptor reflexes may inhibit the medullary respiratory center. Another factor may be the narcotic action of CO<sub>2</sub>. It is probable that, though 20-30 per cent CO<sub>2</sub> may be needed for the production of surgical anesthesia, concentrations in the 10.4 per cent range may produce less marked but definite cerebral and medullary depression.

Hardgrove, Roth and Brown (11) stated that 10 per cent CO<sub>2</sub> could be inhaled for five minutes without ill effects. Only two of our 31 subjects and none of Brown's (9) were able to tolerate 10.4 per cent CO<sub>2</sub> for five minutes because of dyspnea, headache, dizziness, faintness or fainting. Our and Brown's concentration of CO<sub>2</sub> was 0.4 per cent higher than Hardgrove et al.'s and this may be a partial explanation. The recumbent position in Hardgrove's group may also have aided in the tolerance to CO<sub>2</sub> inhalation.

Our experiments indicate that after high concentrations of CO<sub>2</sub> were breathed for short periods of time (2.5 to 10 min.), CO<sub>2</sub> was eliminated rapidly from the body since the respiratory minute volume returned to normal within 3 minutes (average). Such rapid elimination does not always occur, however, if the blood CO<sub>2</sub> has been elevated for hours instead of minutes or if the blood pressure is low instead of abnormally high (12).

An opportunity was afforded in these experiments to note the level of minute volume at which the subjects noted dyspnea. Several subjects breathing more than 100 liters per minute insisted that they had no dyspnea, though they noticed augmented respirations. This re-emphasizes that dyspnea bears no consistent relation to the minute volume of respiration. Some subjects did not even notice augmented respiration (hyperpnea) when breathing 30-40 liters per minute.

Barcroft and Margaria (13) noted upon themselves that the hyperpnea following inhalation of CO<sub>2</sub> was considerably less than that produced by severe exercise. Our findings upon a much larger series confirm their observations and also their conclusions that the small changes in arterial pCO<sub>2</sub> occurring during muscular exercise cannot be an explanation for the hyperpnea of muscular exercise. It is believed that many factors contribute to the increased ventilation of exercise, of which maintenance of arterial pCO<sub>2</sub> at near-normal levels is only one (14).

Attention should be called to the fact that subjects performing strenuous muscular exercise usually do not breathe more than 66 per cent of the maximal breathing capacity, even at a time when their working muscles have incurred an oxygen debt. The muscles therefore are not able (either by activation of stretch receptors or by elaboration of chemical substances locally or into the general circulation) to increase respiration to a maximum. This failure of

respiration to increase to maximal capacity may be due to fatigue of the respiratory muscles (this is not an important factor in the maximal breathing capacity test which lasts for only 30 sec.) or to the presence of inhibitory factors. The rise in blood pressure associated with strenuous exercise may be such a factor by inhibitory reflexes aroused in the carotid sinus and aortic arch pressure receptors. The failure of respiration to increase further may also be due to the fact that fatigue of the leg muscles rather than dyspnea was the factor which limited the severity and length of the exercise.

The rapidity of fall in diastolic blood pressure at the termination of the  $\text{CO}_2$  inhalation at a time when systolic blood pressure and respiration have returned only partially toward normal indicates a sudden decrease in peripheral resistance. It is known that  $\text{CO}_2$  has a direct vaso-dilator effect on peripheral vessels, which is masked by a stimulant effect upon the medullary vasoconstrictor center. The abruptness of fall in diastolic blood pressure suggests a sudden withdrawal of a vasoconstrictor reflex rather than a gradual decrease in stimulant amounts of  $\text{CO}_2$  acting upon the vasomotor center. Whether such a reflex could arise in the respiratory passages in response to irritant concentrations of  $\text{CO}_2$  or in the chemoreceptors in response to abnormally high levels of arterial  $\text{pCO}_2$  is a matter for speculation. It has been suggested that the sudden reduction of high arterial or alveolar  $\text{CO}_2$  tensions may play a part in the production of "cyclopropane shock", in which systolic and diastolic blood pressure may fall abruptly at the termination of long periods of depressed respiration and  $\text{CO}_2$  accumulation (15).

#### SUMMARY AND CONCLUSIONS

The respiratory and circulatory responses of normal young men to inhalation of 7.6 and 10.4 per cent  $\text{CO}_2$  were measured.

When 7.6 per cent  $\text{CO}_2$  in oxygen was inhaled (42 subjects) the average minute volume of respiration increased to a maximum of 51.5 liters per minute (range 24 to 102), pulse rate increased by 16.7 beats per minute and blood pressure rose 30.8 mm.Hg systolic and 22.2 mm.Hg diastolic.

When 10.4 per cent  $\text{CO}_2$  in oxygen was inhaled (31 subjects) the average maximal minute volume rose to 76.3 liters per minute (range 40 to 130), pulse rate increased 15.6 beats per minute and blood pressure rose 33.4 mm.Hg systolic and 25.0 mm.Hg diastolic.

When the  $\text{CO}_2$  inhalation was stopped, respiration and systolic blood pressure returned slowly to normal; diastolic blood pressure fell abruptly upon removal of the mask, often to lower than control figures.

A comparison was made in 19 subjects of the maximal ventilation produced by *a*, inhalation of 7.6 per cent  $\text{CO}_2$ ; *b*, inhalation of 10.4 per cent  $\text{CO}_2$ ; *c*, exhausting muscular exercise, and *d*, maximal voluntary hyperventilation; the average figures were 48.9, 71.4, 109.6 and 166 liters per minute, respectively. The reasons for the failure of the body to respond with greater hyperpnea to high concentrations of  $\text{CO}_2$  and severe muscular exercise are discussed.

Data bearing upon the following are included: 1, the times for which 7.6 and 10.4 per cent  $\text{CO}_2$  are tolerated by healthy men; 2, the symptoms produced

by these concentrations, and 3, the degree of hyperpnea at which dyspnea was noted by normal subjects.

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# DISTURBANCES IN THE CHEMISTRY AND IN THE ACID-BASE BALANCE OF THE BLOOD OF DOGS IN HEMORRHAGIC AND TRAUMATIC SHOCK<sup>1</sup>

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Gregersen and Root (1) have demonstrated that the signs which are shown by man in traumatic shock can be reproduced experimentally in dogs. In these animals the blood volume is decreased 30 to 40 per cent and the cardiac output is reduced 60 to 85 per cent (2). The slowing of the blood flow is accompanied by a decrease in O<sub>2</sub> consumption, an increase in the RQ and by certain changes in the chemical composition of the blood, such as an increase in lactate concentration and a decrease in the CO<sub>2</sub> content and capacity which indicate profound disturbances in tissue metabolism. This led us to make a detailed study of the electrolyte changes in the blood during traumatic and hemorrhagic shock. The experiments were performed between November 1942 and December 1943.

**METHODS.** Ordinary mongrel dogs, weighing between 8 and 20 kgm., were used. The animals were not fed after noon of the day preceding the experiment, but they had free access to water until they were placed on the animal board.

**Trauma.** The thigh muscles were contused with a light raw-hide covered mallet, as described elsewhere (1). After this procedure, which took some 15 to 20 minutes, the administration of ether was discontinued.

**Hemorrhage.** Thirty to 40 per cent of the previously determined blood volume was removed by bleeding the animal from the femoral artery through a cannula inserted under local anesthesia (2 per cent novocain).

**Criteria of shock.** As described in a previous report (1) the animal's condition was followed throughout the experiment by observing his response to external stimuli, the degree of coldness of the extremities, the color of the mucous membranes, the appearance of superficial blood vessels, the rectal temperature, the heart rate and the femoral arterial blood pressure.

**Blood volume.** Plasma volume was determined with the blue dye, T-1824 (see 1). Hematocrit readings were taken on heparinized blood in Wintrobe tubes. The total blood volume was calculated from the formula:

$$\frac{\text{plasma volume}}{100 - (\text{hematocrit} \times 0.96)} \times 100$$

in which the factor 0.96 is the correction for the trapping of dye-tinged plasma between the packed erythrocytes. It may be noted that recent evidence sup-

<sup>1</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

ports the validity of this method of calculating blood volume from plasma volume and the hematocrit value (4).

*Electrolyte determinations.* Femoral arterial blood samples were drawn into oiled syringes which had previously been rinsed with heparin solution. Aliquots were transferred immediately to precipitating fluids for the determination of whole blood lactate and pyruvate. The remaining blood was centrifuged and the plasma removed for further analyses. Additional arterial blood samples were withdrawn for pH and  $\text{CO}_2$  measurements. Since heparin introduces an error in the determination of total base, sulfate and calcium, blood for these analyses was collected in syringes containing no heparin and allowed to clot under oil.

*Total base* was determined by the electro dialysis method of Keys (5).

*Sodium and potassium.* Plasma was dry-ashed in silica beakers in a muffle furnace at  $450^\circ\text{C}$ . Sodium was analyzed by the uranyl zinc acetate method of Butler and Tuthill (6) and potassium was estimated by the chloroplatinate technique of Shohl and Bennett (7).

*Calcium* was determined according to the Clark and Collip modification (8) of the Tisdall method.

*Magnesium.* After precipitation of calcium, an aliquot of the supernatant fluid was used for the analysis of magnesium which was precipitated as magnesium ammonium phosphate as described by Haled (9). The magnesium was calculated from phosphate which was estimated by the colorimetric method of Fiske and Subbarow (10), adapted for making the readings in the Wedge photometer with a 680 mu eyepiece filter.

The *pH* of whole blood and of plasma was determined immediately at  $38^\circ\text{C}$ , using a hypodermic type of glass electrode (11). The accuracy was tested several times during each experiment with appropriate buffer solution and was found to be  $\pm 0.01$  pH unit.

The  *$\text{CO}_2$  content* of whole blood or plasma was estimated according to the technique of Van Slyke and Neill (12). The whole blood  $\text{CO}_2$  was measured on most of the animals and in these instances the plasma  $\text{CO}_2$  was calculated using the Van Slyke-Sendroy line chart (13). Since this chart was constructed for use with human blood, it seemed necessary to determine whether the same assumptions could be made for dog blood. For this reason, estimations of whole blood  $\text{CO}_2$  and of plasma  $\text{CO}_2$  were carried out on 6 dogs in which the whole blood  $\text{CO}_2$  varied between 12.1 and 35.6 volumes per cent (table 1). The plasma  $\text{CO}_2$  calculated from the line chart factor agrees to within  $\pm 0.7$  volume per cent (average 0.08) with the determined plasma  $\text{CO}_2$ . The results demonstrate that the plasma  $\text{CO}_2$  of dog's blood can be estimated accurately from the pH, the hematocrit value and the whole blood  $\text{CO}_2$ .

The *plasma bicarbonate* was calculated from the plasma  $\text{CO}_2$  content and the blood pH, using the  $\text{pK}'$  value of 6.1 given by Peters and Van Slyke (14, formula 6, p. 881).

*$\text{pCO}_2$ .* The  $\text{CO}_2$  tension of the arterial blood was calculated from the pH and the  $\text{CO}_2$  content of arterial blood, using formula 5 on page 881 of Peters and Van Slyke (14).

*Plasma proteins* were estimated from the refractive index according to the method of Neuhausen and Rioch (15) or by the micro-Kjeldahl method (16).

*Base bound by protein* was calculated from equation 12 on page 778 of the paper by Van Slyke et al. (17).

*Lactate.* Whole blood and plasma lactates were determined by the method of Friedmann, Cotonio and Shaffer (18), using an apparatus similar to that designed by West (19). Since a constant ratio was found between plasma and whole blood lactates, the plasma lactate was calculated from the whole blood lactate in a few experiments.

*Pyruvate.* Whole blood pyruvate was determined according to the modification of the method of Lu made by Bueding and Wortis (20). The alkali and bicarbonate were adjusted to determine total keto acids as pyruvate. The concentration of plasma keto acids was assumed to be that of the whole blood.

TABLE 1

*The relation of the plasma CO<sub>2</sub> calculated from the pH and whole blood CO<sub>2</sub>, by means of the Van Slyke-Sendroy line chart (13) to the determined plasma CO<sub>2</sub>*

ANIMAL	DETERMINED WHOLE BLOOD CO <sub>2</sub>	DETERMINED PLASMA CO <sub>2</sub>	CALCULATED PLASMA CO <sub>2</sub>
	vols. %	vols. %	vols. %
Dog 1, control; pH 7.34, hct. 43.4.....	35.6	43.0	42.8
Dog 2, 2 hrs. post transfusion; pH 7.23, hct. 28.7....	34.7	44.6	43.9
Dog 3, 2 hrs. post transfusion; pH 7.36, hct. 41.0....	24.9	30.1	29.7
Dog 4, shock; pH 7.23, hct. 28.7.....	23.1	25.2	25.7
Dog 5, shock; pH 7.19, hct. 43.3.....	13.1	14.7	15.4
Dog 6, terminal shock; pH 7.04, hct. 44.6.....	12.1	13.9	14.1

*Phosphate.* The colorimetric method of Fiske and Subbarow (10) as modified by Guest and Rapoport (21) was followed for the plasma inorganic phosphate determination. Amidol was substituted for amino-naphthol sulfonic acid as the reducing agent (22).

*Plasma water.* The percentage of water in blood and serum was obtained by drying the samples at 78°C to a constant weight.

*Serum chlorides* were done by the absorption indicator method of Saifer and Kornblum (23). The results were frequently checked against those obtained with the method of Van Slyke and Sendroy (24).

*Sulfate* was estimated by the technique developed by Power and Wakefield (25).

The values for the various electrolytes are expressed as milli-equivalents per liter of plasma water.

**RESULTS.** The anion changes which occur after hemorrhage or muscle trauma are shown in tables 2 and 3 which summarize the observations on 10 dogs. The reduction in blood volume varied from 21 to 57 per cent of the control values. The major blood changes in both the hemorrhagic and the traumatic series consisted of decreases in arterial pH, CO<sub>2</sub> tension and bicarbonate concentration and increases in the concentrations of phosphate and lactate.

TABLE 2  
Concentrations (m.eq. per liter of plasma water) of anions and total base in the blood of dogs before (C) and after (S) trauma (T)  
and hemorrhage (H) or both (HT)

Dog no.....	H1		H2		H3		H4		T1		T2		T3		HT1	
CONDITION.....	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Wt., kgm.....	10.0	225	10.2	47	11.6	137	10.3	280	10.1	84	13.4	277	9.4	228	—	186
Time, minutes*		32		57		46		36		—		37		28		21
Blood volume, % reduction....																
Arterial pH.....	7.28	7.28	7.31	7.21	7.32	7.25	7.32	7.29	7.36	7.19	7.40	7.32	7.40	7.28	7.40	7.23
Plasma H <sub>2</sub> O, gms./L.....	946	957	941	952	926	934	912	917	928	932	923	919	933	933	925	923
pCO <sub>2</sub> , mm. Hg.....	37.0	27.9	39.9	13.4	40.1	26.9	42.2	6.8	43.7	14.7	37.4	21.9	34.7	31.8	30.0	20.0
Cl, m.eq.....	110.9	113.2	113.1	113.4	108.0	108.0	107.4	109.2	115.3	116.2	113.0	115.8	113.6	115.7	114.6	118.5
HCO <sub>3</sub> , m.eq.....	17.9	13.4	20.6	5.5	23.0	12.4	23.0	3.5	25.6	6.3	24.4	11.9	22.4	13.5	19.7	8.6
Base bound by protein, m.eq....	12.8	11.0	14.6	13.7	14.7	12.5	20.7	14.9	14.9	12.7	16.6	16.3	13.6	12.2	15.6	13.2
Lactate, m.eq.....	2.3	6.0	2.3	8.2	3.9	9.0	3.7	14.6	2.5	18.8	2.1	8.3	2.1	8.0	2.5	12.2
HPO <sub>4</sub> + H <sub>2</sub> PO <sub>4</sub> , m.eq.....	2.6	4.5	1.4	9.7	1.5	7.7	2.0	9.1	2.3	7.5	1.9	4.5	1.2	4.4	1.8	5.8
Pyruvate, m.eq.....	0.1	0.5	0.1	0.4	0.1	0.5	0.2	0.5	0.1	0.5	0.1	0.4	0.2	0.5	0.2	0.7
Total base, m.eq.....	161.2	168.7	165.4	167.3	168.6	168.1	158.6	162.5	168.8	172.6	168.5	174.8	165.0	167.8	168.6	169.9
Sum of anions, m.eq.....	146.6	148.6	152.1	150.9	151.2	150.1	157.0	151.3	160.7	162.0	158.1	157.2	153.1	154.3	154.4	159.0
B - A, m.eq.....	14.6	20.1	13.3	16.4	17.4	18.0	1.6	10.7	8.1	10.6	10.4	17.6	11.9	13.5	14.2	10.9
Cl <sub>e</sub> /Cl <sub>p</sub> .....	0.61	0.64			0.45	0.52	—	0.69	—	—	—	—	—	—	0.51	0.63

\* Time after hemorrhage or trauma.

The pH of the control arterial bloods varied from 7.28 to 7.42, averaging 7.35. During shock the pH ranged between 6.92 and 7.35. It was lower in those animals which, after surviving for many hours, eventually died. In one animal not recorded in the tables, a terminal value of 6.8 was found.

The bicarbonate of the control arterial blood averaged 22.0 m.eq., ranging between 17.9 and 25.6. After hemorrhage or trauma the values decreased. Two-thirds of the determinations made during shock fell between 10 and 15 m.eq., although values as low as 3.5 (table 2, H4) were observed.

TABLE 3

*The progressive changes in the concentrations (m.eq. per liter of plasma water) of anions and total base produced in the blood of dogs by hemorrhage or muscle trauma*

Dog. No.....	T1					H1		
	Control	Shock				Control	Shock	
CONDITION.....								
Wt., kgm.....	20.4					13.0		
Time, min.*.....		65	135	233	318		90	250
Blood, vol. % red.....			37				38	
Arterial pH.....	7.42	7.31	7.35	7.32	7.28	7.33	7.29	6.92
Plasma H <sub>2</sub> O, gm./L.....	946	940	941	941	941	942	947	947
pCO <sub>2</sub> , mm. Hg.....	34.0	27.0	23.5	23.3	22.2	35.6	24.3	27.3
Cl <sup>-</sup> , m.eq.....	116.0	111.7	110.5	113.6	114.8	117.8	115.1	118.3
HCO <sub>3</sub> , m.eq.....	24.0	14.2	13.6	12.6	10.9	19.3	11.0	5.7
Base bound by protein, m.eq.....	14.6	15.3	15.3	15.1	14.8	14.5	12.7	10.7
Lactate, m.eq.....	3.9	7.6	7.2	6.3	7.3	2.7	6.3	9.4
HPO <sub>4</sub> + H <sub>2</sub> PO <sub>4</sub> , m.eq.....	2.2	4.3	4.0	4.9	5.0	1.9	4.3	6.3
Pyruvate, m.eq.....	0.1				0.3	0.1	0.3	0.4
Total base (B), m.eq.....	168.8	161.0	165.0	164.8	165.0	166.6	164.7	173.1
Sum of anions, (A) m.eq.....	160.8	153.1	150.6	152.5	152.8	156.3	149.7	150.8
B - A, m.eq.....	8.0	7.9	14.4	12.3	12.2	10.3	15.0	22.3

\* Time after trauma or hemorrhage.

The control CO<sub>2</sub> tension of the arterial blood averaged 37.8 mm. Hg, varying from 30.0 to 43.7. In every animal the pCO<sub>2</sub> decreased after hemorrhage or muscle trauma. The values obtained in most instances were between 10 and 30 mm. Hg, but in one dog (table 2, H4) the extremely low figure of 6.8 was recorded.

The average phosphate concentration in the control bloods was 1.9 m.eq., ranging between 1.2 and 2.6. After hemorrhage or trauma the phosphate increased, being greater in the hemorrhaged (average 6.3, range 4.3 to 9.7 m.eq.) than in the traumatized dogs (average 4.9, range 4.0 to 6.5 m.eq.).

The lactate concentration of the blood of the control animals varied from 2.1 to 3.9 the average being 2.8 m.eq. After hemorrhage or muscle trauma the blood lactate increased to values of 6.3 to 18.8 m.eq. (average 9.0).

The concentration of the other constituents studied showed little or no change. The pyruvate increased by about 0.3 m.eq. and the base bound by protein

decreased in all but one instance from 0.3 to 5.8 m.eq. The plasma water increased from 5 to 10 parts per 1000 in the bled dogs; it varied from a decrease of 4 to an increase of 6 parts per 1000 in the traumatized animals (tables 2, 3, 4). The chloride concentration showed an average increase of 0.6 m.eq. (range, -3.4 to +2.8). In all but one experiment the total base increased between 1.8 and 7.8 m.eq. The sum of the anions determined decreased in most of the experiments, but the decrease was greater than 1.5 m.eq in only 3 instances.

In every experiment the sum of the anions is less than the total base value. During the control period, the deficit in the different animals varied from 1.6 to 17.4, the average being 11.0 m.eq. After hemorrhage or muscle trauma the deficit was greater (average 14.6, range 7 to 20 m.eq.).

TABLE 4

*Concentration (m.eq. per liter of plasma water) of cations, total base and their differences in the blood of dogs before (C) and after (S) trauma (T) or hemorrhage (H) or both (HT)*

DOG NO.....	H1		H2		T1		T2		T3		T4		HT1	
CONDITION .....	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Wt., kgm.....			10.3		10.2		10.2		8.3		9.0			
Time, minutes.....		230		260		174		84		129		103		186
Blood volume, % reduction.....		29		36		33		37		35		24		21
Plasma H <sub>2</sub> O, gms./L.	930	939	912	917	932	930	926	930	930	929	915	921	925	923
pCO <sub>2</sub> , mm.Hg.....	46.5	33.3	42.2	6.8	39.1	12.9	41.5	17.9	42.4	22.5	34.1	25.7	30.0	20.0
Na, m.eq.....	159	162	149	148	156	155	156	155	158	156	156	158	158	157
K, m.eq.....	4.38	7.71	4.73	7.62	4.78	6.30	6.17	6.50	5.08	5.72	5.10	5.08	7.30	5.70
Ca, m.eq.....	6.62	5.44	5.86	5.84	5.12	5.37	5.64	5.28	5.75	5.06	5.48	5.17	5.70	5.70
Mg, m.eq.....	2.05	2.74	1.44	2.85	2.19	3.22	2.04	2.58	1.78	2.74	2.02	3.02	2.00	1.90
Sum of cations (C), m.eq.....	172.1	177.9	160.9	164.5	167.6	169.2	169.8	170.0	170.4	169.8	168.8	170.8	173.0	170.4
Total base (B), m.eq.....	172.5	171.0	158.6	162.5	165.7	171.0	166.8	167.3	172.0	170.8			168.6	169.9
B-C, m.eq.....	0.4	-6.9	-2.3	-2.0	-1.9	1.8	-3.0	-2.7	-1.6	1.0			-4.4	-0.5

\*Time after hemorrhage or trauma.

Dog HT appears in table 2 with the same symbols; dog H2 appears in table 2 as dog H4.

The progressive nature of the anion changes which follow hemorrhage or muscle trauma is shown in table 3. In one animal (T1) the blood volume was reduced by 37 per cent by muscle trauma and in the other (H1) the volume was decreased 38 per cent by hemorrhage. These animals have not been selected to compare the effects of hemorrhage with those produced by trauma, but to illustrate the relation of elapsed time after reduction of the blood volume to the changes in anion concentrations which have been described.

The effect of hemorrhage or muscle trauma upon the concentrations of the individual cations is presented in table 4. The sodium and calcium concentrations were not changed by hemorrhage or trauma. The potassium concentration of the 2 dogs which were bled increased 3.33 and 2.89 m.eq. In the 4 traumatized animals changes varied between -0.02 and +1.52 m.eq. The average for the 7 dogs of table 4 was an increase in potassium of 1.02 m.eq. The magnesium concentration increased about 1 m.eq.

The sum of the cations agreed well with the total base determinations. In

dog H1 the sum of the cations was 6.9 m.eq. greater than the total base during shock and in dog HT1 the sum of the cations was 4.4 m.eq. greater than the total base of the control. The differences in the other experiments lie within the experimental error of the determinations. The increase in the sum of the cations and the total base concentrations which occurred during shock can be accounted for by the increase in the concentrations of potassium and magnesium.

**DISCUSSION.** The major changes in the arterial blood electrolytes after hemorrhage or muscle trauma consist of decreases in the pH and  $\text{CO}_2$  content and increases in the concentrations of lactate and phosphate. Essentially the same changes have been reported by Richards (26) in 4 cases of human traumatic shock.

In the experiments on muscle trauma the dogs were anesthetized with ether during the 15 to 20 minutes required to traumatize the muscles. Unfortunately, ether anesthesia produces blood electrolyte changes of the same nature as those which follow muscle trauma. For example, in dogs one hour of ether anesthesia decreases the  $\text{CO}_2$  capacity by 4.8 to 6.6 mM. (27). The effect of ether upon the acid-base balance was eliminated in a series of 7 dogs by using animals in which the spinal cord had been severed between the tenth and twelfth thoracic segments two weeks or more before the experiments were carried out. In 3 of the spinal dogs the lumbar sympathetic chains were also removed. Since the hind legs of these animals were without sensation, the thigh muscles could be contused in the absence of general anesthesia. Comparison of the results obtained on such animals with those reported in tables 2 and 3 shows that regardless of whether comparison is made on the basis of the averages of the two groups or comparison is made between individual animals in which the blood volumes and the times after injury are the same, the group or individual dog which received no ether showed a greater decrease in pH and arterial  $\text{CO}_2$  content and a greater increase in lactate concentration than was observed when ether was administered. Although this result is probably fortuitous, it indicates that the ether anesthesia exerted no additive effect upon the acid-base changes induced by muscle trauma.

The decrease in arterial  $\text{CO}_2$  after muscle trauma or hemorrhage has been reported by several investigators (26, 28, 29, 30). The change in arterial  $\text{CO}_2$  can be accounted for only in part by the increase in the concentration of blood lactate which we and others (26, 29, 30, 31) have found. Since the increase in lactate fails to account completely for the decrease in blood  $\text{CO}_2$ , the presence in increased concentration of other fixed acids must be considered.

Our data (tables 2 and 3) show that after muscle trauma or hemorrhage the plasma inorganic phosphate also increases. Similar observations have been reported following muscle trauma produced by hammer blows (32, 33) or by tourniquet (34). Allison et al. (35) have investigated the phosphate relations of the plasma and red cells in traumatized and bled dogs. In these animals the whole blood phosphate increases as a result of a rise in inorganic phosphate in both plasma and cells. Since the concentration of organic acid-soluble phosphorus in the red cells either increases or remains unchanged, it is clear that the increased inorganic phosphorus does not come from this source. Breakdown of

phosphocreatine following muscle injury is probably partially responsible for the hyperphosphatemia, for the plasma creatine is also increased under these conditions (32). The finding of a progressive increase in inorganic phosphate both in traumatic shock and in hemorrhagic shock suggests that factors other than direct muscle injury are involved. The increased phosphate may be related to decreased excretion, for we and others (32) have observed an increased blood NPN after hemorrhage and muscle trauma, and decreased renal function is characteristic of shock (36, 37).

Other anion changes of somewhat smaller magnitude occur after hemorrhage or muscle trauma. Our experiments show an increase in the plasma pyruvate of approximately 0.3 m.eq. A similar elevation of the pyruvate concentration was noted by Govier and Greer (38) in hemorrhaged dogs. The base bound by protein decreases with decreasing pH. Some uncertainty is attached to these values for they were calculated using a formula derived from experiments on human blood (17).

In agreement with Manery and Solandt (39), we find that dogs in shock show little or no change in the plasma chloride concentration. Richards (26), on the other hand, reports that in cases of trauma the plasma chloride concentration is slightly increased. Although in our experience the plasma chloride is essentially unchanged, the chloride concentrations of the red cells increases, causing an increase in the ratio of the chloride in the cells to the chloride in the plasma (see table 2).

The failure of the total base (B) determinations to agree with the sum of the anions (A) suggests the presence of some undetermined anion. The average difference between these values (B-A) was 11.0 m.eq. in the control animals and 14.6 m.eq. after hemorrhage or muscle trauma. In an attempt to account for the B-A deficit we have determined the plasma sulfate in a series of 15 dogs before and after reduction of the blood volume. The average control sulfate value was 2.2 m.eq. After hemorrhage or muscle trauma there is a gradual rise in sulfate concentration, the magnitude depending upon both the degree of injury and the time after injury at which the blood sample was taken. The maximal values obtained 6 to 7 hours after reduction of the blood volume averaged 4.4 m.eq. When the B-A values are corrected for sulfate, the control deficit is 8.4 and that after injury becomes 10.1 m.eq. In patients in shock the B-A value is said to vary between 8 and 11 m.eq. (26). These results suggest that some unaccounted for fixed acid is present.

In addition to the increased concentrations of fixed acids, the plasma bicarbonate during shock is reduced by the increased pulmonary ventilation which occurs after hemorrhage or muscle trauma (2, 26, 28, 30). The excessive respiration reduces the  $p\text{CO}_2$  and, therefore, the plasma bicarbonate concentration. The nature of the acid-base changes is shown by plotting the pH,  $p\text{CO}_2$  and the arterial plasma  $\text{CO}_2$  content in Peters' logarithmic form (14, p. 915). The plot of the data obtained from dog H3 of table 2 shows a curve (fig. 1) falling in the area of metabolic acidosis, partly compensated by the decrease in  $p\text{CO}_2$ .

The total base agrees well with the sum of the cations (table 4) in both control and shocked dogs. Of the several cations determined potassium deserves special comment. A number of investigators have reported that the plasma potassium is elevated after muscle trauma (29, 39, 40, 41). Holmes (40) who has made a careful study of potassium during shock finds that immediately after muscle trauma the serum potassium level is unchanged. On the other hand, Gutmann et al. (29) noted an increase of 43 to 84.5 per cent in the concentration of plasma potassium at this time. All investigators find a considerable terminal

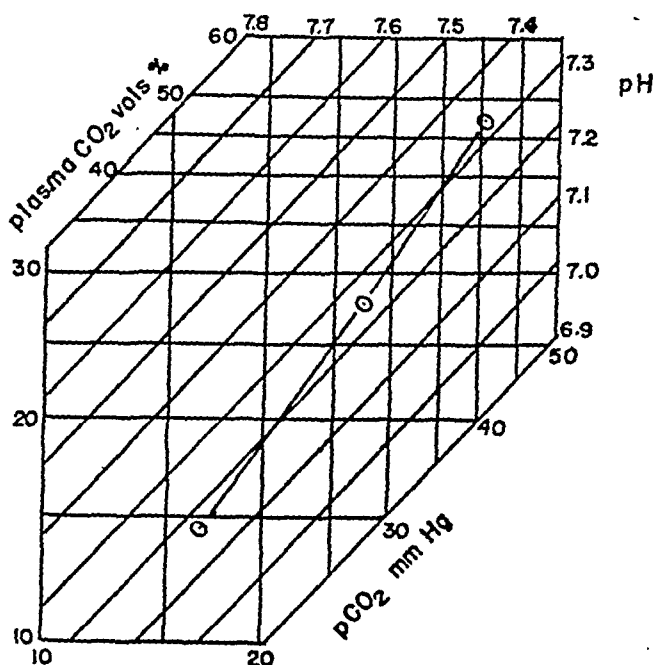


Fig. 1. Acid base chart of dog H 3 (table 2) showing displacement into the region of metabolic acidosis by the removal of 46 per cent of the blood volume. The control arterial blood pH was 7.32 and the CO<sub>2</sub> content was 47.3 vols. per cent. One hundred and thirty-seven minutes after the animal was bled the pH was 7.25 and the CO<sub>2</sub> content, 27.3 vols. per cent. One hundred and thirty-nine minutes later the pH had decreased to 7.18 and the CO<sub>2</sub> content was 14.7 vols. per cent. Over-ventilation decreased the pCO<sub>2</sub> from a control value of 40.1 to 26.9 mm. Hg one hundred and thirty-seven minutes after the hemorrhage and to 16.8, two hundred and seventy-six minutes after bleeding.

rise in potassium. Holmes' (40) values ranged from 8 to 13 m.eq. Manery and Solandt (39) state that immediately preceding death an increase of 100 to 200 per cent was observed. In only 2 of 13 animals did Gutmann et al. (29) observe potassium values approaching the level of 14.16 mM. per liter which Winkler, Hoff and Smith (42) state exerts a toxic effect on the heart. In a more recent study Winkler and Hoff (41) find that after tourniquet shock the concentration of serum potassium seldom exceeds 8 mM. per liter, a level at which they noted only minor changes in the electrocardiogram. According to these authors, it is exceptional for death to be caused by cardiac arrest resulting from potassium autointoxication. After respiratory failure, there may be a rapid agonal ele-

vation of serum potassium. The results indicate that it is only in terminal samples that the potassium may reach toxic levels. We do not know the significance of the slight increase in magnesium which follows hemorrhage or muscle trauma. The sodium and calcium values are essentially unchanged.

Comparison of the changes in the canine blood constituents which follow hemorrhage with those which are found after muscle trauma reveals only minor differences between the two conditions. Thus, for the same decrease in pH, dogs which are bled show a larger increase in phosphate than do traumatized animals (see 35). In both man (30) and dog (2), trauma produces a greater increase in pulmonary ventilation than occurs after hemorrhage. This results in a lower arterial  $p\text{CO}_2$  after trauma than is shown following hemorrhage. The striking changes in blood constituents are the consequence of a reduction in blood volume—a factor common to hemorrhage and muscle trauma.

With the exception of an increase in the plasma chloride concentration reported in patients in shock, the nature of the changes in the blood electrolytes in response to muscle trauma are the same in our dogs as those observed in man (26). However, the changes seen in the dog are greater than those observed in man. There are several reasons for this. No therapeutic measures were undertaken in the animals investigated, whereas in the studies on man infusions of one sort or another were commonly carried out. Consequently, the blood samples were drawn from animals under conditions of more profound shock and in some dogs terminal samples were studied. Moreover, the well-known excellence of the canine respiratory and circulatory systems must have prolonged the duration of the shock state and, since time is an important factor in the development of metabolic acidosis, thus led to the large changes which we have found. The volume of blood removed for the various determinations constituted a greater proportion of the dog's blood volume than was the case in human studies and in some instances probably shortened the period of survival.

Among the various theories concerning the nature of traumatic shock is the conception that "toxic" substances are liberated by the injured tissues (28). In connection with this hypothesis it should be realized that the complex metabolic disturbances which are indicated by the changes in the blood constituents during traumatic shock may in themselves constitute a "toxic factor", the results of which must be clearly distinguished from any injurious effects that may be ascribed to "toxins liberated from tissues at the original site of injury".

Analysis of our data shows a significant correlation in every experiment between the decreased pH and the increase in lactate, pyruvate and phosphate. The correlation is strikingly illustrated by plotting increases in phosphate against decreases in pH. Furthermore, when phosphate is plotted against per cent reduction in blood volume, it is apparent that the increase in phosphate occurs when the blood volume is decreased in excess of 20 per cent, and that it rises progressively to higher values in animals in which the reduction is greater than this (35).

The changes in the blood constituents indicate the nature and the extent of some of the metabolic disturbances which occur when the blood flow is slowed

by decreasing the blood volume. Measurements of the arterial pH,  $\text{CO}_2$ , lactate and phosphate, therefore, provide further quantitative criteria not only for determining the depth or degree of shock, but also for testing at various stages the effectiveness of transfusion or any other therapeutic procedure (see 43).

#### SUMMARY

The major changes in blood chemistry and in acid base balance which occur when the blood volume of dogs is reduced by hemorrhage or muscle trauma consist of progressive decreases in pH and arterial  $\text{CO}_2$  content and progressive increases in the concentrations of lactate and phosphate. There are slight increases in pyruvate and sulfate, and the base bound by protein is reduced. Little or no change takes place in plasma water and chloride concentrations. In the control animals the sum of the anions is less than the total base values. This deficit becomes larger during shock, suggesting an increase in some unknown fixed acid. The metabolic acidosis present during shock is partly compensated by a reduction in the arterial  $\text{CO}_2$  tension.

The sum of the cations ( $\text{Na} + \text{K} + \text{Ca} + \text{Mg}$ ) agrees well with the total base determinations. The increase in the sum of the cations which occurs after hemorrhage or muscle trauma is accounted for by increases in the concentrations of potassium and magnesium.

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# THE EFFECT OF INTRAVENOUS ADRENALIN ON BLOOD FLOW, SUGAR RETENTION, LACTATE OUTPUT AND RESPIRATORY METABOLISM OF PERIPHERAL (LEG) TISSUES IN THE ANESTHETIZED CAT

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The calorogenic action of adrenalin for the mammalian organism as a whole has long since been indubitably established. In contrast with this certainty, however, the locus of this increase in respiratory metabolism must still be regarded as in doubt. This is not due to lack of effort, but to the conflicting nature of the evidence on record. This is too voluminous to be reviewed here; suffice it to say that in attempts to determine whether the hormone is a specific and general stimulant to cellular metabolism nearly all organs and tissues of the mammalian body and some others have been tested in vivo and/or in vitro. These include the adrenal gland itself, blood cells, brain, heart, kidney, liver, nerve trunks, placenta, pituitary, sarcomas, skeletal and smooth muscle, skin, thyroid, tumors and yeast. Also, mere separation into a predominantly visceral or peripheral locus has been attempted by use of hepatectomized or eviscerated preparations, or of single limbs. The variable reports as known to us include: 52 that can be interpreted as indicating general stimulation; 24 describing inhibition; 29 indicating no effect or at least none in the absence of the liver; and 11 suggesting that the effect depends on the concentration of adrenalin, or of available oxygen or the presence or absence of functioning sympathetic nerves.

Our own previous experience has been negative: no effect could be observed on isolated frog muscles (Griffith, 1923); and the oxygen utilization of the hind leg following intra-arterial injection of adrenalin was reduced (Griffith and Hummel, 1930; Cammer and Griffith, 1939); but, since the rate of blood flow was, also, it was concluded that this was the specific effect of adrenalin and the reduced respiratory metabolism was merely secondary to it. In these experiments, use of intra-arterial injection had been resorted to in the effort to isolate any possible specific, peripheral action. The result being negative would not have to mean, however, that the peripheral tissues do not ordinarily participate in the normal calorogenic response to adrenalin administered intravenously or entering the blood stream from the glands themselves. Although it has been agreed since the work of Boothby and Sandiford (1923) that the calorogenic action of adrenalin is something more than any possible specific dynamic action of glucose resulting from the carbohydrate plethora afforded by the accompanying hyperglycemia, it was hypothetically not inconceivable that adrenalin might be unable to exert this effect in the absence of an increased tissue sugar supply. Or, the hypertension resulting from its usual mode of action might overcome the

constrictor effect which usually resulted from intra-arterial injection and so increase rather than decrease peripheral blood flow, thus making possible an increased peripheral oxygen consumption. Or, again, it might be possible that some, as yet, entirely unsuspected visceral adjuvant available only when adrenalin was free to act viscerally as well as peripherally was necessary for the peripheral effect itself.

For these reasons, it was decided to study the metabolism of a hind leg during the action of intravenously administered adrenalin; and this was extended to include not only oxygen consumption and carbon dioxide output, but sugar retention and lactate output as well; all being computed from measurement of blood flow and arterial-venous differences corrected for any change in blood hydration.

**PROCEDURE.** Cats under nembutal-urethane anesthesia were prepared for intravenous injection (jugular vein), arterial blood sampling (carotid artery) and for securing a sample of venous blood and determining its rate of flow from a hind leg; this last being done as follows. The left leg was always retained for observation and its circulation, therefore, left intact. The right iliac artery was ligated merely to prevent engorgement and loss of blood into this leg when the iliac vein from it was subsequently cannulated. This cannula, of large bore to prevent obstruction by clotting and permit easy cleaning, was placed centrally in the right iliac vein and as close to the vena cava as a protecting bull-dog clamp permitted. All other branches into the vena cava, except the left iliac, for two centimeters or so above its origin, were ligated.

A venous blood sample from the observed left leg was obtained and its rate of flow determined by transfer of the clamp protecting the cannula in the right iliac vein to the vena cava about one centimeter above its origin, thus diverting all blood from the left leg into the cannula. From this it was taken up as rapidly as it appeared by syringe, the filling of which was carefully timed. A previous report (Griffith et al., 1946) offers a defense for the retention of this primitive, but thoroughly dependable measurement of flow rate so long after the development of others of apparently superior elegance.

As much of this venous sample as required for analysis was retained and the remainder immediately reinjected.

While this venous sample was being obtained a simultaneous arterial sample was secured from the carotid artery.

Adrenalin chloride solution (Parke-Davis) was diluted just before use with isotonic NaCl to provide the rates of administration shown in the tables when injected intravenously (jugular) 1 cc. per minute. Injection was for 5 minutes; it was begun immediately after determination of the normal rate of blood flow and obtaining a pair of normal, simultaneous arterial-venous (carotid-iliac vein) blood samples; blood flow was again measured and blood samples taken during the last minute of the injection while it continued without change or interruption, or immediately upon its termination.

Heparin was used to prevent clotting. Blood sugar and lactate determina-

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tions were made on Folin-Wu tungstic acid filtrates by the methods of Hagedorn and Jensen and of Friedemann, Cotonio and Shaffer, respectively. Blood hydration was estimated by the colorimetric method of Cohen and Smith as modified by Wu for alkaline hematin. Oxygen and carbon dioxide contents were obtained by the manometric method of Van Slyke and Neill; proper precaution was taken to prevent alteration of gas content of the blood samples during collection and while awaiting analysis.

**RESULTS.** *Blood hydration.* The oxygen capacity of venous blood was 0.14 vol. per cent greater than arterial as an average of all determinations; i.e., there is a slight concentration of the blood on passage through the tissues capillaries.

TABLE 1

*The effect of 5-minute intravenous injection of adrenalin on the rate of blood flow in the hind leg of anesthetized cats*

ADRENALIN INJECTION	NUMBER OF DETERMI- NATIONS	BLOOD FLOW	
		Normal	Effect of injection (% of normal)
<i>mgm./kgm./ min.</i>		<i>cc./min.</i>	
0.0005	39	17.5	144
0.001	40	18.6	138
0.002	40	16.4	117
0.004	44	14.4	108
0.008	49	15.3	103

TABLE 2

*The effect of 5-minute intravenous injection of adrenalin in the anesthetized cat on arterial blood lactate level, and the rate of output of lactate by the tissues of the hind leg into the blood*

ADRENALIN INJECTION	NUMBER OF EXPERI- MENTS	BLOOD LACTATE*		
		Change in arterial blood lac- tate con- centration (mgm. %)	Effect on tissue lac- tate output	
<i>mgm./kgm./ min.</i>			<i>mgm./ min.</i>	Per cent of normal
0.0005	7	-0.6	+1.46	356
0.001	7	+1.5	+0.35	142
0.002	10	+1.9	+0.25	126
0.004	8	+2.5	+0.43	156
0.008	17	+3.6	+0.37	137

\* Average normal arterial blood lactic acid concentration for the 49 experiments, 14.3 mgm. per cent; average normal output, 0.86 mgm./min.

Following the intravenous injection of adrenalin this concentration effect is slightly diminished, venous blood being then only 0.10 vol. per cent greater in average oxygen capacity than arterial. These values, both normally and after injection are almost exactly the same as those found in the previous work (Griffith et al., 1946) in which adrenalin was administered intra-arterially rather than intravenously. There was no observable relationship between the dosage of adrenalin, within the range employed here, and the effect on blood hydration. And although, as a matter of principle, corrections were made in accordance with the indicated change in hydration, these were negligibly small and had no effect on the average results or their interpretation.

*Blood flow.* The data of table 1 indicate that the average effect of adrenalin administered intravenously at the rates and under the experimental conditions

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employed here is to increase to a greater or less extent the rate of flow of blood through a hind leg. Since arterial blood pressures were not recorded it is impossible to be certain as to the factors involved; but from generally accepted premises it is not improbable that the maximal increase with the minimal rate of injection implies the lack of any local vasoconstriction and perhaps some dilatation since arterial blood pressure probably was raised very little and might be expected occasionally to fall. As the rate of administration is increased, a balance between increasing degrees of local vasoconstriction and elevation of arterial pressure would explain the progressive decline in the augmentation of blood flow as observed here, until with maximal rate of injection the two practically offset each other, and flow rate remains essentially unaltered.

It deserves to be stressed that either qualitatively or quantitatively these results are perhaps not safely transferable to other experimental conditions. A large amount of unpublished plethysmographic work supports suggestions already in the literature that adrenalin may affect the circulation in the limb quite differently under different anesthetics or in the absence of anesthesia altogether. And even under the routinely standardized procedure of these experiments the effect of any given rate of injection was unpredictable in any one cat or in the same cat from one injection to another (see tables 3 and 6). In particular, if initial blood pressure is low, adrenalin even in high concentration seemed to improve the circulation in the leg more than otherwise; but in addition to this there must be other factors, at present uncontrollable, influencing results to the end that those described here are to be considered only as a valid description of the average reaction under the special conditions of these experiments.

*Lactic acid output.* These results (tables 2 and 3) permit no doubt that adrenalin may increase lactate output by the peripheral tissues of the leg; and although this was the average effect with all rates of injection it was not invariable: once there was no change and in 14 there appeared to be a decrease—these occurring with about equal frequency at all rates of administration (table 3).

In addition, these data present a curious challenge to interpretation. It will be noted (table 2) that with all except the lowest rate of adrenalin injection, arterial blood lactate concentration is increased, and progressively with dosage as might be expected if adrenalin specifically augments lactate liberation by tissue (muscle) cells. With the lowest rate of adrenalin administration, however, arterial lactate concentration is actually slightly reduced. In both of these respects these data conform very well with those of a previous report on adrenalin lactacidemia (Griffith et al., 1939). This independent confirmation of this seemingly paradoxical reduction of arterial lactate concentration with the lowest rate of adrenalin injection makes it less easy than formerly to dismiss it as possibly an experimental error. Any physiological explanation of it, however, would appear only to be made increasingly difficult by the seemingly equally anomalous maximum increase of lactate output by the leg tissues by this same minimal rate of adrenalin administration. The only reconciliation of these apparently contradictory effects which comes to mind is the possibility that, arterial concentration

TABLE 3

*Correlation of changes of lactate output with change of blood flow of the hind leg of anesthetized cats following 5-minute intravenous injection of adrenalin*

ADRENALIN INJECTION	CHANGE IN BLOOD FLOW (CC./MIN.)							
	>-3	-3 to -1	-1 to 0	0	0 to +2	+2 to +5	+5 to +10	>+10
mgm./kgm./min.	Change in lactate output (mgm./min.) with the no. of expts. in parenthesis							
0.0005	—	—	(1) -0.26	—	(1) +0.57	(1) +0.63	—	(4) +1.28
0.001	—	(1) +0.45	—	—	(2) -0.32	(1) +1.02	—	(3) +0.42
0.002	(2) -0.24	(3) +0.03	(2) +0.19	(1) -0.02	—	—	(1) +0.05	(1) +2.09
0.004	(2) +0.17	—	(2) +0.58	(1) +1.48	(1) 0.00	(2) +0.38	—	—
0.008	(5) +0.06	(1) +1.33	(1) +0.68	(1) +0.26	(1) -0.02	(7) +0.45	—	—
Average	(9) +0.01	(5) +0.37	(6) +0.33	(3) +0.57	(6) -0.02	(11) +0.48	(1) +0.05	(8) +1.06
	(9) +0.01	(11) +0.35		(9) +0.17		(11) +0.48	(9) +0.95	

TABLE 4

*The effect of 5-minute intravenous injection of adrenalin on arterial blood sugar level, arterio-venous blood sugar difference and the rate of sugar uptake from the blood by the tissues of the hind leg of the anesthetized cat*

ADRENALIN INJECTION	NUMBER OF EXPERIMENTS	BLOOD SUGAR				
		Change in arterial blood sugar concentration (mgm. %)	Arterio-venous difference (mgm. %)		Effect on tissue sugar uptake	
			Normal	After injection	Mgm./min.	Per cent of normal
mgm./kgm./min.						
0.0005	9	+18	10	11	+2.41	179
0.001	10	+23	10	18	+3.91	240
0.002	10	+70	11	46	+8.27	482
0.004	14	+85	13	68	+8.43	487
0.008	19	+60	15	48	+7.94	321

representing merely a balance between input and outgo, some other organ, possibly liver, is stimulated under these conditions to remove lactate from the blood even slightly faster than it is being added to it. But if this is so, another

problem immediately presents itself: why, with much less augmentation of output from the peripheral tissues by the remaining rates of adrenalin injection does arterial lactate concentration actually increase? Could it be at all reasonable to assume that as adrenalin dosage increases, liver (?) activity in removing lactate from the blood is reduced either by vasoconstriction with reduced access to liver cells or through some specific adrenalin effect?

The association of maximum increase of lactate output with maximum increase of blood flow supports Cori's persistent contention (Cori et al., 1935) that adrenalin lactacidemia is not asphyxial in origin, but the result of a specific cellular action; for, as argued above, there is no reason to doubt that the large increase of blood flow with the minimal rate of adrenalin administration must be due to lack of any considerable constriction and possibly even to local vasodilatation. In this one of its activities, however, the remaining augmentations of lactate output would, at least superficially, appear to indicate that unlike other of its effects (vasoconstriction; cardiac acceleration; intestinal inhibition) this one is not proportional to adrenalin concentration. This may be due only, however, to the fact that demonstration and quantitation of this particular effect could be seriously and, in experiments of this kind, unavoidably interfered with and obscured by concomitant vasomotor effects. Perhaps the maximum output with the lowest dosage does not mean that whatever specific effect adrenalin has on lactate liberation is maximum at this concentration of it in the blood, but that the accompanying vasodilatation permits maximum access to the tissue cells with maximum transport of any lactate liberated. As adrenalin concentration increases, its effect on those cells to which it is accessible might increase proportionally while total measured tissue output was diminished through vasoconstriction. Indeed this seems to be the only explanation which would account for the almost uniform augmentation of measured output at all but the minimum rate of adrenalin administration: a (hypothetical) progressively intense lactate liberation almost exactly balanced by progressive restriction of the areas of tissue formation by a (very probable) progressive intensity of vasoconstriction.

Correlation of the lactate output with change of blood flow is more clearly revealed in table 3 where the data of table 2 are rearranged specifically in relation to concomitant change in blood flow.

*Tissue sugar retention.* It may be seen in table 4 that the uptake of sugar from the blood by the tissues of the leg is being greatly augmented during the 5th minute of adrenalin hyperglycemia. Indeed this was the most constant effect, aside from the hyperglycemia itself, in this series of determinations: as has been mentioned the effects on blood flow and lactate output were variable; and, as will be seen, the effect on oxygen utilization and carbon dioxide output is extremely so; in contrast, however, sugar retention was invariably increased. Much and often as the hyperglycemia has been invoked as an essential mechanism in the calorigenic response to adrenalin, this, to our knowledge, is the first direct measurement of its relation to and effect upon tissue sugar uptake.

In this relationship it is seen that both hyperglycemia and tissue retention increase to a maximum with injection at the rate of 0.004 mgm./kgm./min., but that a rate of administration greater than this (0.008 mgm./kgm./min.) results in reduced increases of each. This reversal of effect in so far as the hyperglycemia is concerned provides welcome confirmation of the only previous description of it (Griffith et al., 1939), but does little to advance explanation, except to indicate that increased tissue sugar "utilization", then surmised as one of several possible factors, is less than likely to be responsible; for increase of tissue sugar uptake is seen now to be reduced correspondingly. This latter no doubt could be related to the intensification of vasoconstriction which had to be postulated to explain the failure of this highest rate of injection to have any significant effect on blood flow in spite of the undoubted maximal elevation of arterial blood pressure. But, if reduced increase of blood flow and thereby of total tissue sugar supply were the cause of the reduced increase of retention, hyperglycemia should be increased. The reduced hyperglycemia following maximal adrenalin injection must, therefore, be due to reduced blood sugar augmentation.

Being thus forced to conclude that the reduced increase of retention with the highest rate of adrenalin administration is probably due to reduction of the diffusion gradient established by the blood sugar level, directs attention to the latter as probably the controlling factor throughout. This is reinforced if comparison is made of the effects of 0.0005 and 0.004 mgm./kgm./min.: with the former, and using the average figures of tables 1 and 4, blood flow is increased 7.6 cc./min. and blood sugar level, 18 mgm. per cent, which would increase the amount of sugar brought to the tissues by 1.4 mgm./min.; with the higher rate of administration the corresponding figures are 1.1 cc./min. and 85 mgm. per cent, or an increased tissue supply of only 0.9 mgm./min. Thus with the lowest rate of injection, an increase of supply of 1.4 mgm./min. is accompanied by a 79 per cent increase in tissue sugar uptake; while, with the higher, an increase of supply of only 0.9 mgm./min. is accompanied by an increase of tissue retention of 387 per cent. Or, again, 306 per cent increase in retention (387-79 per cent) accompanies an increase in blood sugar level of  $\left(\frac{85 - 18}{18} \times 100\right)$ , or 372 per cent, while the actual total supply is decreased  $\left(\frac{1.4 - 0.9}{1.4} \times 100\right)$ , or 36 per cent.

For total supply to have been determinant in the amount of sugar retained by the tissues from the blood would imply an equilibrium between the latter and metabolic utilization by them. This as far as we know would, at least in the main, be either immediate oxidation or synthesis to glycogen, either or which would entail increased oxygen utilization. In the absence of any measurable effect on the latter as described in the following section, it is fortunate to be able to invoke a purely physical equilibrium with no metabolic connotations and in which the diffusion gradient established by blood sugar level appears to be the chief controlling factor in so far at least as concerns the quiescent metabolic condition of an anesthetized animal.

*Respiratory Metabolism. Oxygen consumption.* The significance of the data of tables 5 and 6 can be fully appreciated only if it is recalled that the rates of adrenalin administration employed here are such as invariably increase the respiratory metabolism of the animal as a whole (Griffith et al., 1940; Jones and Griffith, 1945). These results would indicate that this calorogenic action may occasionally receive contribution from, but is not dependent upon the peripheral tissues; those of table 5 make it quite clear that the average, over-all effect of

TABLE 5

*The effect of 5-minute, intravenous injection of isotonic NaCl and of adrenalin on oxygen consumption and carbon dioxide output of the tissues of the hind leg of the anesthetized cat*

INJECTION	NUMBER OF DETERMI- NATIONS	NORMAL	AFTER INJECTION	EFFECT OF INJECTION	
				Mean difference	MD* PEoD
				cc./min.	
Oxygen consumption					
0.9 % NaCl	15	0.84 ±0.07	0.93 ±0.08	+0.09 ±0.11	0.8
Adrenalin (mgm./ kgm./min.)					
0.0005	30	0.97 ±0.05	0.90 ±0.06	-0.07 ±0.08	1.0
0.001	30	1.02 ±0.06	0.89 ±0.04	-0.13 ±0.07	1.9
0.002	30	1.07 ±0.07	1.02 ±0.07	-0.05 ±0.10	0.5
0.004	30	0.81 ±0.05	0.65 ±0.05	-0.16 ±0.07	2.3
0.008	30	0.89 ±0.04	0.96 ±0.04	+0.07 ±0.06	1.1
Carbon dioxide output					
0.9 % NaCl	15	0.78 ±0.06	0.82 ±0.11	+0.04 ±0.13	0.3
Adrenalin (mgm./ kgm./min.)					
0.0005	30	0.94 ±0.06	1.24 ±0.11	+0.30 ±0.13	2.3
0.001	30	0.83 ±0.08	1.07 ±0.11	+0.24 ±0.14	1.7
0.002	30	1.13 ±0.07	1.28 ±0.12	+0.15 ±0.14	1.1
0.004	30	0.61 ±0.06	0.70 ±0.06	+0.09 ±0.08	1.1
0.008	30	0.78 ±0.05	0.94 ±0.06	+0.16 ±0.08	2.0

\* Critical ratio: Mean Difference divided by the Probable Error of the Difference.

adrenalin at any rate of injection is statistically insignificant, in that the mean differences are in no case as much as three times their probable errors; and, further, insofar as there tends to be any effect at all, it is predominantly inhibitory. This last would appear from table 6 to be an accompaniment of concomitant reduction in blood flow. At least, with but one insignificant exception, all reductions of blood flow are accompanied by roughly proportionate decreases in oxygen utilization as shown in the left-hand side of the table. The simplest explanation of this would seem to be that under the conditions of these experi-

ments with intravenous administration of adrenalin at rates that are predominantly pressor in action, reduced blood flow could only rarely be attributed to reduced arterial pressure and must generally have resulted from an intensity of local vasoconstriction sufficient to counteract an accompanying hypertension.

It will also be noticed that a reduction of oxygen utilization occurs as fre-

TABLE 6

*Correlation of changes of oxygen consumption and carbon dioxide output with change of blood flow of the hind leg of anesthetized cats following 5-minute intravenous injection of adrenalin*

INTRA- VENOUS ADREN- ALIN	CHANGE IN BLOOD FLOW (CC. PER MIN.)															
	-20 to -3		-3 to -1		-1 to 0		0		0 to +2		+2 to +5		+5 to +10		+10 to +41	
	Change in CO <sub>2</sub> output and O <sub>2</sub> consumption (cc. per min.) with the number of experiments in parenthesis															
	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
mgm./ kgm./ min.																
0.0005	(1)		(6)		(8)		(1)		—		(4)		(6)		(4)	
	+1.11	-0.70	-0.18	-0.21	-0.30	-0.06	+0.05	-0.02	—	—	+0.52	-0.07	+0.78	+0.22	+0.84	-0.09
0.001	(5)		(2)		(3)		—		(6)		(5)		(3)		(6)	
	-0.10	-0.26	-0.65	-0.13	-0.14	-0.04	—	—	-0.02	+0.01	+0.26	-0.19	-0.19	-0.19	+1.38	-0.13
0.002	(5)		(6)		(4)		—		(4)		(2)		(5)		(4)	
	-0.72	-0.53	-0.04	+0.01	-0.10	-0.07	—	—	-0.07	+0.10	+1.11	+0.30	+0.01	-0.10	+1.64	+0.29
0.004	(4)		(4)		(6)		(3)		(4)		(3)		(2)		(4)	
	-0.04	-0.30	-0.27	-0.24	-0.01	-0.15	-0.03	-0.01	-0.14	+0.07	+0.48	-0.22	+0.87	-0.05	+0.61	-0.36
0.008	(3)		(5)		(3)		—		(8)		(6)		(4)		(1)	
	-0.76	-0.35	0.00	-0.12	+0.14	-0.06	—	—	+0.02	+0.05	+0.54	+0.24	+0.52	+0.66	+0.46	+0.11
Average	(18)		(23)		(24)		(4)		(22)		(20)		(20)		(19)	
	-0.30	-0.38	-0.16	-0.14	-0.12	-0.08	-0.01	-0.01	-0.04	+0.05	+0.51	+0.01	+0.40	+0.14	+1.11	-0.03
	(65)						(85)									
	-0.18		-0.17 ± 0.05		+0.47						+0.03 ± 0.05					

quently as an increase even when blood flow is augmented. This probably results from the two different means whereby blood flow may probably be increased under the conditions of these experiments: viz., 1, local (muscular?) vasodilatation; and 2, a hypertension sufficient to drive an increased amount of blood through still patent channels in spite of extensive local vasoconstriction. The first, by increasing access to previously anemic areas might be expected to

result in increased oxygen utilization; the second, equally with reduced blood flow, as above, could be expected to be accompanied by a reduction of oxygen utilization.

This would seem to provide a satisfactory explanation of these results in terms of the known vasomotor effects of adrenalin and without assuming any specific effect of it on oxygen utilization by the peripheral tissues. Further work may show it to be incomplete; and it is true that these results can be explained equally well by assuming adrenalin increases or decreases oxygen utilization with either effect antagonized or abetted by accompanying vasoconstriction or dilatation. But until further work shows that a specific effect of either kind is involved, the simpler hypothesis seems preferable. At all events the main question these experiments were designed to test seems to have been answered, to the effect that the calorogenic action of adrenalin for the animal as a whole is contributed to only infrequently by increased oxygen utilization by the peripheral tissues and usually occurs in spite of their oxygen utilization being diminished.

*Carbon dioxide output.* Again, as with oxygen consumption, it may be recalled that the rates of intravenous adrenalin administration employed here invariably increase the total carbon dioxide output of the animal as a whole. By contrast, the data of table 5 indicate that the average, overall effect for these peripheral tissues is statistically insignificant, although, in contrast to oxygen consumption, whatever trend there is, is positive rather than negative. This positive trend as well as its average insignificance is shown by table 6 to be due to the occurrence at all rates of injection of positive and negative results (with the former, on the average, predominating) roughly proportional to the concomitant change in blood flow. Thus, reduction of blood flow, with only two exceptions, is accompanied by reduced carbon dioxide output, which, on the average ( $-0.18$  cc./min.) is very similar to the average reduction of oxygen consumption ( $-0.17$  cc./min.). Increased blood flow is accompanied more often by increased carbon dioxide output (16 out of 21 instances) than by increased oxygen consumption (10 out of 21) and the increase of the former (av.,  $+0.47$  cc./min.) is much greater than that of the latter (av.,  $+0.03$  cc./min.) which is altogether insignificant. Also, this excess of carbon dioxide output over oxygen utilization is especially marked with the largest increases of blood flow. From these facts it may be inferred that, on the whole, carbon dioxide output is related to blood flow in the same manner as oxygen utilization as analyzed in the previous section; but that it is additionally augmented by displacement with lactic acid under the conditions of increased blood supply which favor increased release of it as described in a previous section.

*DISCUSSION.* The effect of intravenously administered adrenalin on blood flow through the hind limb or resting muscle of anesthetized dogs has been studied by Mertens and Kahlson (1936), Rein (1937), Mertens and Rein (1938) and by v. Issekutz (1941); according to the first three, it is decreased; according to the last, it is increased; in our own work reported here, either effect may occur with any rate of injection and even in the same animal at different times. This lability of vascular effect is undoubtedly related to the variable effect on lactate

output and oxygen consumption. Unlike Kramer and Schäfer (1939) we do not find that increased output of lactic acid is proportional to reduced oxygen consumption and occurs only with vasoconstriction; rather, in this and a preceding report on the effect of intra-arterial injection of adrenalin (Griffith et al., 1946) lactate output was increased maximally with maximal increases of blood flow and vasoconstriction of any severity suppressed it. Nor do we support Rein (1938) or v. Issekutz (1941) to the effect that adrenalin only depresses the oxygen consumption of the resting leg. Again, as with lactate output, the effect is variable: it is reduced if blood flow is, as reported by Rein; and may be reduced even if blood flow is increased as found by v. Issekutz; but in the latter instance it may also be increased—a diversity of response which we have attempted in a previous section to relate to the possible dual manner in which blood flow in the peripheral tissues may be increased following intravenous administration of adrenalin.

It might be considered anomalous that oxygen consumption of the peripheral tissues is not more regularly increased in view of the invariable increase in sugar retention by them. This apparently hitherto neglected datum turns out to be, with the accompanying hyperglycemia, the most constant of the adrenalin effects here measured. Even with the greatest reduction of blood flow, sugar retention is increased. And with the persistent (Soskin, 1927) and even intensified (Wilhelmj et al., 1938) specific dynamic action of glucose in the eviscerate or liverless preparation indicating a peripheral locus, it might have been anticipated that the oxygen consumption of these peripheral tissues would have shown a more regular increase in response to the hyperglycemic carbohydrate plethora. It is not improbable that this may have contributed to such increases as were observed, but the evidence implies that usually whatever augmentation may have resulted from this cause is more than offset by the concomitant vasoconstriction and ischemia. This conflict would probably not arise in experiments such as those of Soskin and of Wilhelmj, just referred to, since infusions of glucose solution alone have been found (unpublished experiments), at least in the intact animal, not only to cause no reduction in peripheral blood flow, but, actually, to increase it enormously; probably due to the induced hydremic plethora and its attendant circulatory adjustments. Nor would it arise in the conditions of emergency activity in which adrenalin is normally and physiologically active, since Mertens et al. (1936), Rein (1937) and Rein and Schneider (1930) have shown that adrenalin does not counteract the vasodilatation and may even augment the already increased blood flow in active muscles.

Finally, what may be concluded as to a possible specific effect of adrenalin, either inhibitory or augmentatory, on the metabolic rate of peripheral tissues? Apparently, nothing conclusive. As already argued, the results seem compatible either with a theory of: 1, an adrenalin stimulation which is countered by vasoconstriction and abetted by dilatation; or 2, an inhibition abetted by constriction and countered by vasodilatation; or 3, a lack of any specific effect, but with oxygen consumption paralleling the vasomotor action. The last being the simplest seems preferable until convincing evidence against it is at hand. All that is

proven is that during the action of intravenously administered adrenalin the oxygen usage of peripheral tissues may either increase or decrease. It may be that those (McIver and Bright, 1924; Hunt and Bright, 1926; Cori and Buchwald, 1931) who have still obtained a calorogenic response after hepatectomy or evisceration benefited from the first, and those who were unsuccessful (Crile and Rowland, 1922; Caskey and Humel, 1927; Soskin, 1927; Gatzanjuk, 1937) encountered the second of these possible results.

#### SUMMARY

At the end of 5-minute intravenous (jugular) injections of adrenalin at rates of 0.0005, 0.001, 0.002, 0.004 and 0.008 mgm./kgm./min. the oxygen capacity of venous blood from a hind leg which is normally 0.14 vol. per cent greater than the arterial blood entering it, is still 0.10 vol. per cent greater; i.e., blood hydration is only negligibly affected. The rate of blood flow through the leg may be increased or decreased by any rate of injection but on the average was increased by all and maximally by the smallest and least by the largest rate. Lactate output by the tissues of the leg was increased on the average by all rates of injection, maximally by the least and much less, but approximately to the same degree, by all the other rates of administration; also maximally with the greatest increases of blood flow (the three lowest rates of injection) and not at all on the average if flow rate is decreased more than 3 cc./min. Glucose retention by the tissues of the leg was invariably increased whether flow rate was increased or decreased; maximal retention followed administration of 0.004 mgm./kgm./min., which also produced the maximum hyperglycemia. In spite of this invariable carbohydrate plethora and usual increase of lactate output there is no evidence that oxygen utilization is stimulated; it is actually decreased (av.,  $-0.17 \pm 0.05$  cc./min.) if blood flow is decreased; it is occasionally increased (10 out of 21 instances) when flow is increased, but the average ( $+0.03 \pm 0.05$ ) is negligible. Carbon dioxide output behaves similarly except that it is increased much more (av.,  $+0.47$  cc./min.) than oxygen utilization when flow rate is increased, probably due to lactic acid displacement. Neither carbohydrate plethora nor increased lactate output appear, therefore, to involve an increase in respiratory metabolism of the leg tissues; and adrenalin augmentation of this for the animal as a whole (calorogenic action) only occasionally received augmentation from these peripheral tissues and must usually occur despite them.

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# FACTORS AFFECTING THE ELECTRICAL POTENTIAL OF THE GASTRIC MUCOSA<sup>1</sup>

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Mond (5) in 1927 measured the electrical potential of the frog's stomach and found values between 10 and 70 millivolts. He noted that the longer the time spent in the preparation of an experiment and the more handling and trauma incurred by the stomach during the procedure, the greater was the tendency for the resting potential to be low. Using a vascular perfusion apparatus he caused temporary reductions in the value of the potentials by administration of various salt solutions, while atropine, acetylcholine and adrenalin were without effect. Also  $H^+$  ion increases in the perfusion fluid caused a reduction in the potential. He stated that within the lumen of the stomach only the actual osmotic tension of solutions, regardless of their nature, had an effect on the gastric voltage and concluded that the salts tested effected the potential most when they were applied through the vascular system.

In 1932 Mislowitzer and Silver (3) found that the potential of cats' stomachs under periton anesthesia sometimes exceeded 100 millivolts. Introducing an intra-gastric electrode through the esophagus and placing a serosal one on the center of the anterior stomach wall they found the potential to be substantially lower in the pylorus than in the fundus and cardia. Contrary to the observations of Mond (5), they found that changes of potential due to presence of solutions in the stomach were not due to simple osmotic effects of the solutions but noticed various potential responses from equal concentrations of chloride, iodine, rhodan and citrate introduced into the stomach. They also observed a reduction of potential from the intravenous injection of adrenalin and histamine though they believed these very active drugs to be relatively ineffective on the potential. Vagal stimulation, and section of the vagi and upper spinal cord had little effect on the potential. On the other hand it was noted that the magnitude of the recordable potentials was indicative of the general state of the animal. That is, with vomiting, pulmonary edema, bradycardia or a fall in blood pressure there was a marked reduction in potential.

Later in conjunction with Rothschild (4) Mislowitzer and Silver found by placing ionic solutions of substances of different absorbability in the stomach, that the potential difference decreased during times of the greatest absorption.

Sarre (11) also working with peritonized cats confirmed the fact that there was a potential difference across the gastric membrane. He found the mucosal

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sur ace negative with respect to the serosa and stated that there was an *increase* in potential when the stomach was stimulated to secrete by the injection of histamine.

Quigley et al. (6) have repeated certain phases of Sarre's (11) work by using the apparatus of Adair and Goodman (1) on the stomach and pouch of unanesthetized Pavlov dogs. The circuit was completed with the extra gastric electrode on the abraded skin. They found the resting potential of the stomach to be from 20 to 30 millivolts, and of the pouch 10 to 20 millivolts. They did not observe any significant change in potential following the subcutaneous injection of 0.5 mgm. histamine or 2 mgm. pilocarpine. Similarly they observed no change in potential when solutions with a pH range of from 1.08 to 9 were introduced into the stomach. Neither did hypertonic, isotonic, or hypotonic NaCl solutions affect the potentials when introduced into the stomach or pouch. However 5 per cent dextrose caused an increase in negativity as did whole milk. Soluble casein was without effect.

Rehm (7) has attempted to relate the electrical energy involved in the stomach with the energy required for acid secretion. Working with dogs he has reported that the stomach can maintain its potential at a constant level despite the fact that the maximum current was being drawn in the external circuit. He devised an apparatus to include a small area of stomach (21 sq. cm.) in a miniature water bath which made possible the measurement of the rate of secretion as well as the potential (8). Using pernostonized dogs, zinc acetate, and saturated potassium chloride electrodes, the potential was decreased to a relatively constant level with a progressive increase in secretion by repeated subcutaneous injections of histamine. Accumulation of secreted acid in the stomach to a degree where the pH dropped to 1.9 to 2.6 did not alter the recorded potential. Solutions of HCl of pH 0.64 to 1.1 applied to the mucosa on the other hand led to a fall of potential which appeared to be due to an effect on the gastric cells rather than a simple electro-physical effect from the interaction of solutions. Since a fall of potential occurred with histamine stimulation even though the secretions were buffered, it appeared that the effect of histamine on the gastric voltage was directly due to the effect of the drug on the cells, and not indirectly due to the accumulation of acid.

Later Rehm (9) demonstrated that an electric current applied to the secreting stomach of pernostonized dogs from serosa to mucosa resulted in an increase in the secretion of HCl. This did not occur in non-secreting stomachs. When current was applied in the reverse direction there was a decrease in secretion together with depression of the potential. When the secretion recovered the potential also recovered with it.

In a recent publication Rehm (10) has presented evidence that the steady potential of the stomach arises somewhere between the submucosa and mucosal surface. It is not possible at present to decide whether the source is in the base or apex of the mucosal cells, or in the circulatory network immediately beneath the mucosal layer. The evidence strongly indicates however that the muscle layers and the muscularis mucosae are not the sources.

Finally Goodman (2), using a Leeds and Northrop special micromax recorder, studied the electrical responses of the stomachs of normal humans, and of subjects with gastric lesions, such as ulcer and carcinoma. A naso-gastric tube was used for the stomach lead, and the other electrode was attached to the abraded skin of the forearm. A standard test was evolved using the potential response to milk as the control measure. Data were presented on the effects of various lesions on the response to milk compared with the normal response, and the results indicated that the changes in voltage resulting from the ingestion of certain substances such as milk might be of diagnostic value.

**APPARATUS.** It was seen at an early stage in these investigations that it was necessary to develop a recording equipment which provided adequate stability and sensitivity, and which faithfully recorded voltage changes as fast as one per second. These conditions were fulfilled in the apparatus developed, which consisted of a push pull, single stage, amplifier operating a high resistance galvanometer, and which recorded on moving photographic paper. A further advantage was obtained by using an amplifier in which the input impedance was high (1 megohm) in that the current drawn from the potential source was negligible. This factor minimized the effect of electrode resistance and placement on the recorded potentials.

**Amplifier.** The amplifier circuit is shown in figure 1. It was a single stage, push pull, pentode, class A design. The tubes used provided economy of operation and had sufficient gain and a low enough plate impedance to match roughly the galvanometer used. The problem of drift or base line instability was dealt with by using a common cathode bias resistor. Drift due to varying radiation and conduction of heat from the tubes to the chassis was practically eliminated by raising the tubes slightly in their sockets so that there was no physical contact between the base of the tubes and the chassis. The galvanometer was connected from plate to plate of the two tubes and brought to a null point (with no potential difference across the input of the amplifier) by adjusting the center tap of a wire wound potentiometer connected to the high voltage source.

The galvanometer was specially constructed and was of d'Arsonval type using two 2 inch permanent magnets of the type used in small loud speakers. The moving coil was wound with 2,000 turns of no. 42 enamel covered wire and had a D.C. resistance of 4,200 ohms. It was suspended vertically by a fine phosphor-bronze wire allowing a linear torque movement. The plate load resistors were chosen to allow adequate gain, suitable electrical damping, and frequencies up to one per second.

Records were taken on slow-moving 5 x 7 inch Velox FO paper, by means of a light beam reflected from a silvered microscope cover slip attached to the galvanometer coil. Another light beam reflected from the galvanometer mirror, and was focussed on a red-glass viewing screen. The latter provided a means of observing the voltages being recorded during the experiments. The paper was spring driven by a metronome motor at a speed that required 50 minutes to expose its full length. A time switch connected to the drive mechanism illuminated the optical slit throughout its length by a separate light source, producing verti-

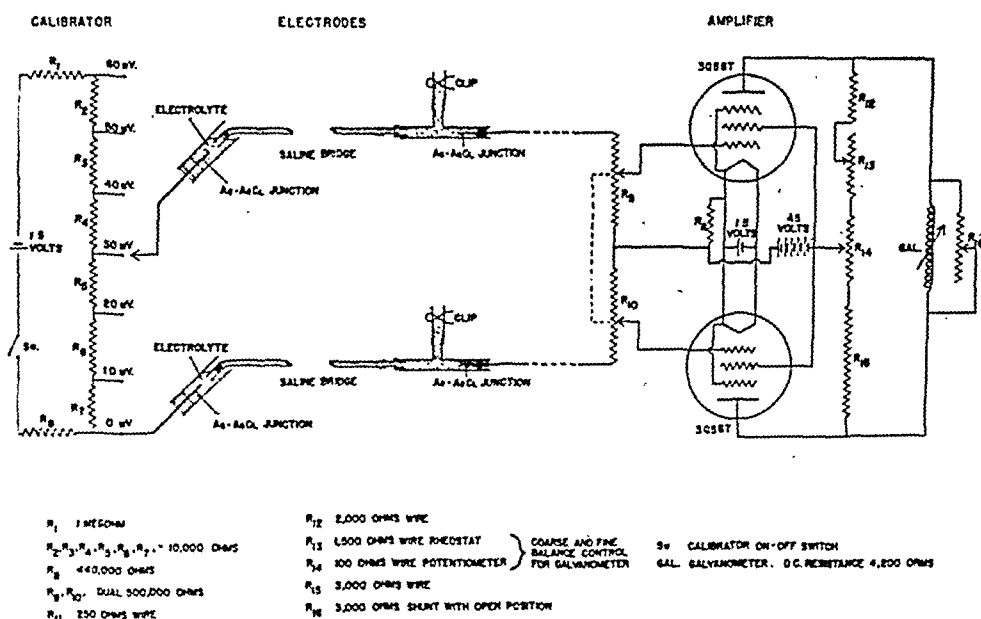


Fig. 1. Schematic diagram of calibrator, electrodes and amplifier

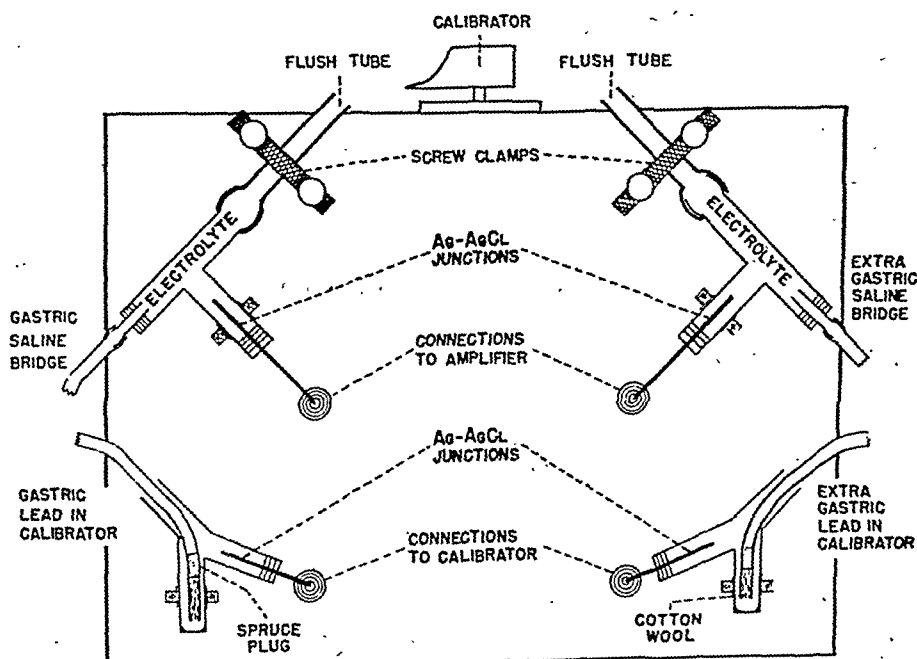


Fig. 2. Detailed diagram of construction and arrangement of electrodes. The mounting was a vertical bakelite plate 10 x 12 inches. The calibrator network was mounted on the back.

cal lines on the record at 10 minute intervals. Procedures were indicated by exposure of a marginal segment of the paper from an independent light source. Maximum gain of the amplifier recording through the saline-bridge electrodes

used in the experiments provided full scale deflection (5 in.) of the light beam with 30 millivolts input. A dual potentiometer in the grid circuits provided any gain required up to the maximum without changing the impedance of the input system.

*Electrodes.* The electrode system consisted of 2 saline bridges connected at one end with the metallic input leads to the amplifier through silver-silver chloride junctions (figs. 1 and 2). Plastic tubing of  $2\frac{1}{2}$  mm. inside diameter was used. This proved to be more satisfactory than the standard naso-gastric tube being more flexible and having a relatively smaller outside diameter for its bore, due to the thinness of the wall. It was therefore easier to insert, especially in humans. It was also unaffected by moisture. The concentration of the electrolyte used was not critical provided it was great enough to permit good conductivity; 10 per cent NaCl proved satisfactory. It was essential however for the electrolyte concentration at the two silver junctions to be identical. A T-tube was incorporated into each saline bridge at the silver chloride junctions to permit replacement of the electrolyte by flushing.

The distal end of each bridge was closed with a  $\frac{1}{2}$  inch spruce plug. This provided a mechanical obstruction to the escape of saline from the tubing but did not decrease the electrical conductivity significantly. This procedure was adopted because it was found in the early experiments that pinching the tubing during its insertion or even the act of swallowing expelled a small amount of electrolyte which was replaced by a bubble of air; a sudden drop in voltage often occurred due to the resulting high resistance of the lead. It was also found necessary to boil the saline when esophageal leads were used, since gas bubbles were driven off in the tubing by the heat of the body if this were not done, and again a high resistance developed in the bridge with a consequent reduction in recorded potential. To ensure that the end of the gastric electrode did not become occluded by end-on pressure against the gastric wall, small windows were cut in the side of the tubing for a distance of 1 inch from the end and distal to the wooden plug. The space in the tip of the tubing was filled with cotton wool moistened with saline. Calibration of the apparatus was carried out by applying potentials of 10 to 60 millivolts from a voltage divider in 10 millivolt steps. The calibrator voltages were applied through the saline bridges from a second pair of low resistance silver-silver chloride junctions connected to the calibrator. The arrangement of the calibrator is also shown in figure 1.

In some experiments gastric secretions and voltages were recorded simultaneously. The secretions were collected in a 2 cc. plastic thimble held in a loop on the arm of a spring balance. The latter was connected with a pivoted mirror which deflected a light beam on to the same paper as used to record the potentials, and thus produced a photographic record of the weight of secretions. The spring balance gave linear displacement of the light beam as the weight of the secretion in the thimble increased from zero. Several thimbles of identical weight were provided so that when one thimble had filled it could be replaced instantaneously by another and the light beam returned to its former base line.

The quantity of secretion is indicated by the total upward excursion of the light beam and the rate of secretion is apparent from the slope of the record. The spring balance was calibrated from time to time with known weights and the actual secretion also weighed in many instances. The photographic tracing provided an accurate record of the weight of the secretion.

A photograph of the recording unit is shown in figure 3.

**PROCEDURE.** The early experiments concerned an effort to record the gastric potentials in humans using the technique outlined by Goodman (2) with slight modifications. This series proved to be unsatisfactory mainly because it was impossible to obtain steady records. There was almost continuous variation in the recorded potentials. It appeared that this was due partly to technical error, e.g., changes in electrical contact with the stomach wall, and partly to physiological effects which were not understood. Therefore a series of experiments on dogs was undertaken to clarify more fully the fundamental factors

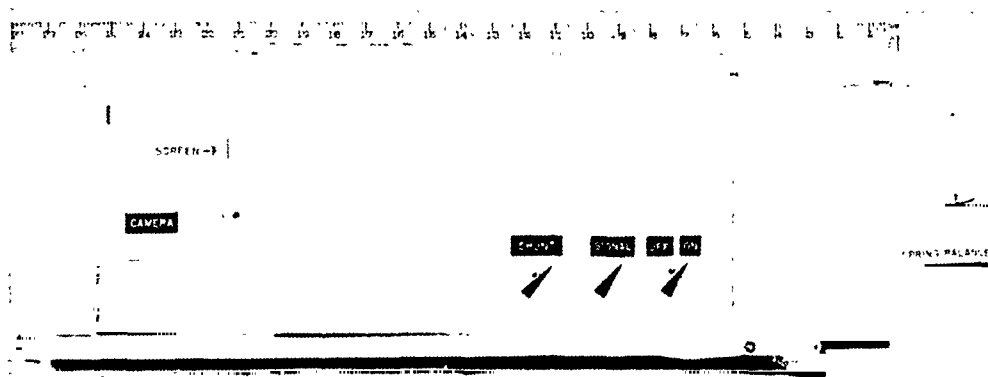


Fig. 3. Photograph of recording unit for gastric potential and secretion, incorporating camera and paper drive mechanism, galvanometer, and spring balance with their optical systems.

affecting the gastric potentials, and to attempt to devise a more reliable technique.

Dogs were used, weighing 4 to 18.5 kilos, the majority weighing 6 to 10 kilos. They were anesthetized with urethane, 1.5 grams per kilo; urethane plus chloralose, 0.75 gram and 40 mgm. per kilo respectively; soluble barbitone, 225 mgm. per kilo; or pentobarbital, 45 mgm. per kilo. Anesthetics were usually given intraperitoneally. The type of anesthetic did not seem to affect the potentials of the stomach, though the depth of anesthesia did to some extent. A tracheotomy was done and a tap cannula inserted into the internal jugular vein for the injection of drugs. The stomach was brought up into a mid-line abdominal incision and a flanged glass gastroenterostomy tube  $1\frac{1}{2}$  cm. in diameter and 15 cm. long was tied into the anterior wall of the stomach by a pursestring suture. All bleeders were caught and tied. The gastrostomy tube protruded through the abdominal incision and was held vertically in a clamp. This tube provided a means of introducing solutions and the recording electrode into the stomach. To prevent salivary secretion from entering and gastric contents from leaving

the stomach, the esophagus was tied in the neck and the pylorus ligated just distal to the sphincter. In tying the pylorus care was taken to exclude the vessels from the ligature.

In the early experiments various electrolytic solutions were put into the stomach into which the gastric electrode dipped. It was found that over a wide range of concentrations the effect of these solutions on the recorded potential difference was insignificant provided they were sufficiently ionized to give good conductivity (see also Quigley, 6). Thus tap water resulted in a slightly lower recorded potential than physiological saline, but there was little difference between 0.9 per cent saline and stronger salt solutions, and various concentrations of hydrochloric acid. Also the gradual increase in acidity that occurred with the accumulation of secreted acid did not alter the background potential significantly. These observations are in agreement with those of Mond (5), Goodman (2) and Quigley et al. (6). Physiological saline was finally adopted as the standard intra-gastric medium. The stomach was filled until the level of saline was half way up the gastrostomy tube. The changing level of the saline in the tube permitted observations on the peristaltic movements and the effect of respiratory movements. The gastric electrode dipping into the saline which filled the stomach recorded the potentials from the mucosa as a whole, rather than from regions of the mucosa. Artifacts due to peristalsis and respiration were almost completely absent.

A second series of experiments was performed in which efforts were made to correlate gastric secretion and potentials. In these cases a gastrostomy opening was made on the anterior stomach wall about 2 cm. proximal to the upper limit of the pyloric antrum. Through this opening a spiral made of 3 mm. glass rod was inserted into the stomach. This served to separate and prevent apposition of the mucosal surfaces so that peristalsis was ineffective, and the steady flow of gastric secretion could be recorded. The size of the glass spiral in each dog was carefully chosen to avoid stretching of the stomach and possible interference with its secretory activity. A straight flanged glass tube 1.5 cm. in diameter to serve as a collecting tube was then tied into the gastrostomy opening with a pursestring suture, and brought out through a skin incision over the right rectus. The dog was placed on its right side, the table top was tilted and the position of the dog and collecting tube adjusted until the long axis of the stomach and the collecting tube were in alignment and almost vertical. The gastric secretions were thus permitted to flow steadily out of the collecting tube. The gastric electrode was inserted through the collecting tube and a cotton wick in the end of it allowed to lie on the mucosa. At the conclusion of the experiment the dog was killed with everything in situ. The accessible (left) flank and lower ribs were carefully cut away leaving the viscera undisturbed. A window was then cut in the fundus of the stomach and the interior of the stomach examined to be sure that there was no obstruction of the flow of secretions and no accumulation of them in the stomach, and to note that the recording electrode made good contact with the mucosa. The position of the electrode was also noted.

In a third series of experiments where it was desired to obtain records of the potentials from various regions of the stomach, the first technique was used, but no saline was placed in the stomach. The electrode was directed through the gastrostomy opening toward the region from which the potentials were to be recorded and the wick in the end of it allowed to rest gently against the mucosa.

The extra gastric lead was placed in various positions. The recorded voltages were not materially affected by its position for it made little difference whether it was placed on the serosal surface of the stomach, in the peritoneal cavity, subcutaneously, or on the moistened surface of the skin. The fact that the site of the extra gastric electrode did not affect the recorded voltages is explained by the high input impedance (1 megohm) of the recording system.

The ends of the gastric and extra-gastric leads were placed together in a beaker of saline before and after each experiment, and the amplifier turned up. If there was any deflection from the zero setting of the galvanometer, a polarization potential existed which was corrected before the experiment by flushing out both bridges with new saline. Only occasionally did a polarization potential develop at the silver-silver chloride junctions during the course of an experiment. It did not exceed 5 millivolts when it did occur.

**RESULTS.** In the initial experiments on humans there was almost continuous fluctuation of the recorded voltage that did not appear to be entirely due to technical artifact. It was observed that the emotional state, and the condition of rest or alertness of the subject materially affected the potential being recorded from minute to minute. A sudden noise, or even the telephone ringing in the next room, or preoccupation with a mental problem, would cause a rapid decrease in the recorded potential. In one subject a sudden startling noise, such as a sharp whistle or a sudden hand clap in the quiet room caused a prompt fall of potential followed by a gradual recovery to a slightly higher than resting voltage, with eventual return to the initial value, the whole sequence occupying about 5 minutes. The records obtained by these methods of stimulation were almost identical, and could practically be superimposed one over the other. Such observations suggested that the fluctuations were physiological and possibly related to changing activity of the sympatho-adrenal system.

*Experiments on Dogs. Background potential differences.* The recorded resting potential difference ranged between 40 and 100 millivolts. It was steady in that it did not show fluctuations or wave patterns, in this respect differing from the human experiments. It did however show a progressive and gradual increase from the beginning of the record for a variable interval of a few minutes to  $\frac{1}{2}$  hour or so. It appeared that this increase represented the gradual assumption of a completely resting condition by the animal following the disturbance created by reflexes aroused during the preparation. The extent of the gradual increase of potential difference until a constant level was attained ranged from no increase to about 10 millivolts. These findings are in accord with those of Mond and Mislowitzer and Silver who found that the gastric potential difference was greatest when the animal was in the resting

state. They are also in accord with our early findings on humans that when the subject was disturbed the potential dropped.

The intragastric electrode was almost without exception negative with respect to the extra-gastric lead. Only as a terminal phenomenon following the death of the animal did the reverse occur, and then the potential difference was slight.

*Effect of adrenalin.* Thirty-nine dogs were given a total of 81 intravenous injections of adrenalin. The results were not modified by the type of anesthetic. The adrenalin dose in each instance was made up freshly into 2 cc. of physiological saline and injected over a period of one-half to one minute. Saline alone injected as a control was without effect on the recorded potentials. The doses of adrenalin ranged from 0.01 to 0.4 cc. of 1:1000 solution. The majority of observations were made with doses of 0.05 to 0.2 cc.

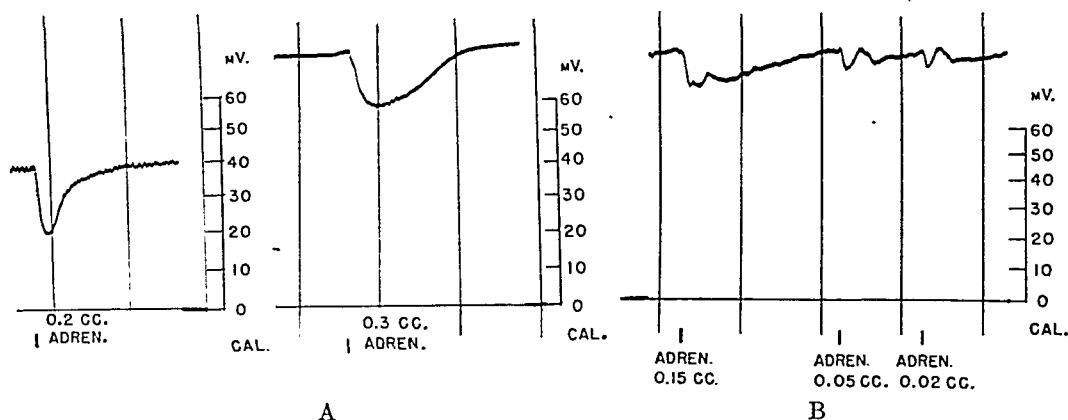


Fig. 4. (Left) Effect of 0.2 and 0.3 cc. 1:1000 adrenalin in two dogs. Simple reduction of potential followed by recovery.

B. (Right) Effect of 0.15 and 0.05 and 0.02 cc. 1:1000 adrenalin in a dog. Biphasic type of responses.

In this and subsequent records vertical lines mark 10 minute intervals.

The responses can be divided into two types. The first type consisted of a prompt fall of potential, usually of 5 to 30 millivolts, attaining its maximum in 1 to 3 minutes, and followed by a fairly steady return to the resting value in the next 3 to 15 minutes (fig. 4A).

The second type of response was typically bi-phasic. It showed a primary drop within one or two minutes, followed by a fairly rapid return toward, sometimes exceeding, the resting voltage, followed by a secondary drop which was usually not as great as the primary fall, with gradual recovery during the next 3 to 20 minutes (fig. 4B). In some instances the response was not clearly bi-phasic but showed a recovery course that was first rapid, and then more gradual, causing a definite angulation in the recovery record. The drop in potential sometimes attained 30 millivolts.

*Effect of pilocarpine on gastric potential.* Forty-five intravenous injections of pilocarpine were given to 25 dogs. The doses ranged from 0.05 to 1.0 mgm.

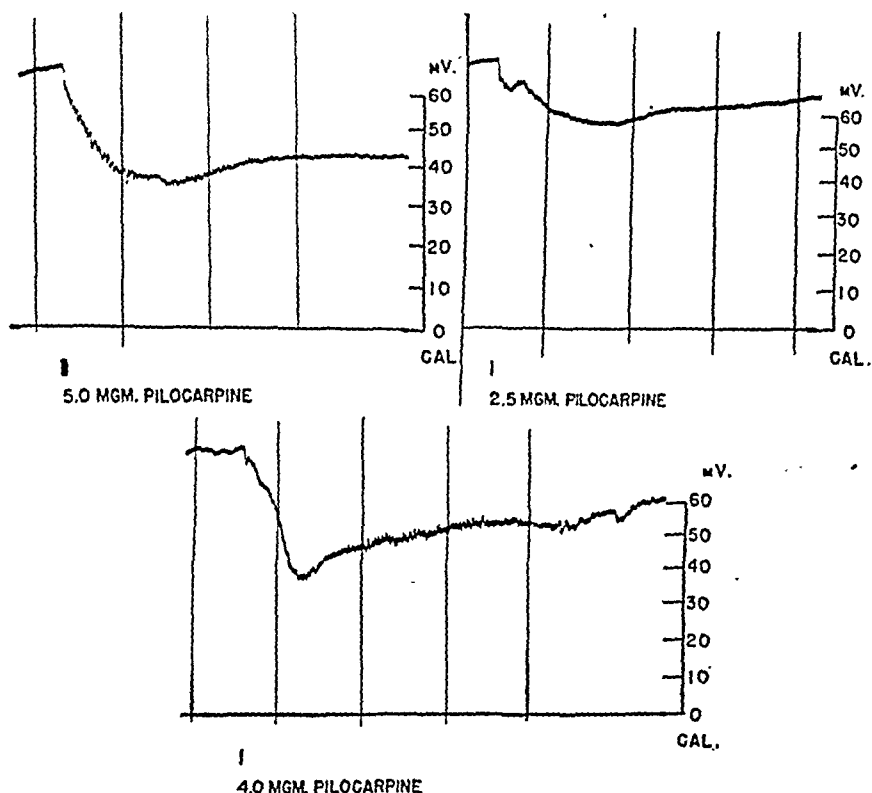


Fig. 5. Effect of pilocarpine in 3 dogs, with doses of 5, 2.5, and 4 mgm. The prolonged effect of pilocarpine is apparent.

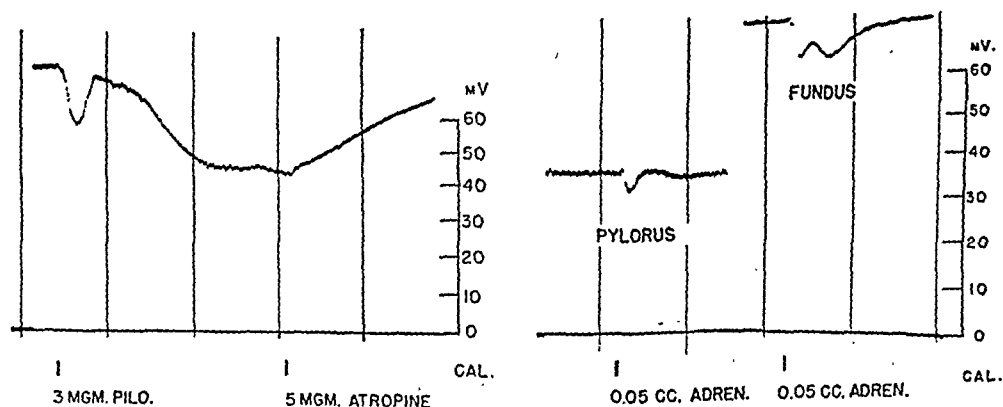


Fig. 6

Fig. 7

Fig. 6. A dose of 3 mgm. pilocarpine caused a typical reduction of potential which was neutralized by atropine.

Fig. 7. Voltage at fundus is approximately twice that at pylorus in the same dog. In each region 0.05 cc. 1:1000 adrenaline caused a biphasic response.

per kilo body weight, made up for injection to 2 cc. volume with physiological saline. The response in practically all cases was typical, the only variation being in degree and duration, which largely depended on the dose. There

was a prompt fall in potential at first rapid then gradually levelling off, and attaining its maximum in 2 to 15 minutes, followed by a gradual recovery to or almost to the original value during the next 15 minutes to  $1\frac{1}{2}$  hours. Occasionally a notch occurred during the fall, which was not always evident. When it occurred it was so typical in form as to suggest a physiological effect, rather than an artifact due to electrode shift. With large doses the potential fell to 50 per cent of the resting value, and on one or two occasions to as low as 25 per cent of the resting value. With small to moderate doses the actual reduction of voltage ranged from 10 to 25 per cent of the resting value (figs. 5, 6 and 10).

*Effect of atropine following pilocarpine.* In 7 instances atropine was injected at varying intervals following the injection of pilocarpine. The doses of atropine were roughly double the dose of pilocarpine. The effect of atropine was not dramatic but in every instance there was evidence that the voltage started to rise more rapidly from the low pilocarpine level than it had been previous to

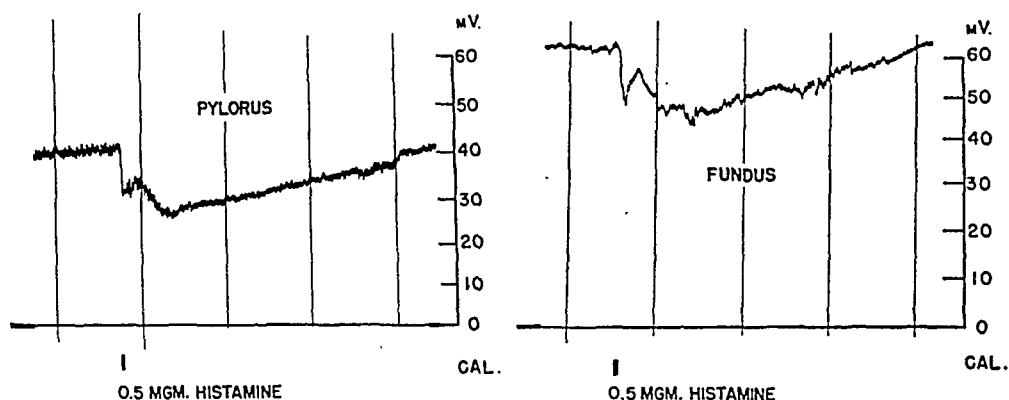


Fig. 8. Voltage at pylorus is approximately two-thirds that at fundus in the same dog. Histamine caused a similar response in each region.

the administration of atropine. In some cases a sharp return toward normal resulted, producing a definite angle in the curve of recovery. In the cases where atropine was given following pilocarpine the voltage returned to the resting value sooner than occurred when similar doses of pilocarpine alone had been given (fig. 6).

*Effect of histamine on gastric potentials.* The effects of intravenous histamine were studied in 24 dogs. Fresh solutions in physiological saline of ergamine acid phosphate were prepared for each experiment. The dosage ranged from 0.026 to 0.12 mgm. per kilo. Different anesthetics were used and did not appear to affect the results.

There was a fairly constant type of response, which varied only in degree and duration with the dose. A prompt decrease of potential occurred following administration of histamine which usually attained its maximum within 1 to 2 minutes of the injection, rarely taking as long as 5 minutes for full development. The potential started slowly to return to normal soon after the full reduction

of potential had been attained. There was occasionally the prolonged low potential more frequently seen with pilocarpine. Return of the voltage to the resting level was usually complete in 20 to 30 minutes. The extent of the voltage reduction varied from 5 millivolts in response to 0.026 mgm. per kilo of histamine to a drop of 20 to 30 millivolts with 0.120 mgm. per kilo. Sometimes a notch occurred at the start of the histamine responses also (figs. 8 and 11).

*Effect of nerve stimulation (femoral and vagus).* Interpretation of the results from electrical stimulation of nerves is difficult. One disadvantage of the high impedance recording system used is that a static charge occurring when the stimulus was applied to the dog sometimes introduced a transient fluctuation in the recorder, which could not be completely eliminated by grounding the animal. Since the potential changes in the stomach are slow and prolonged it would proba-

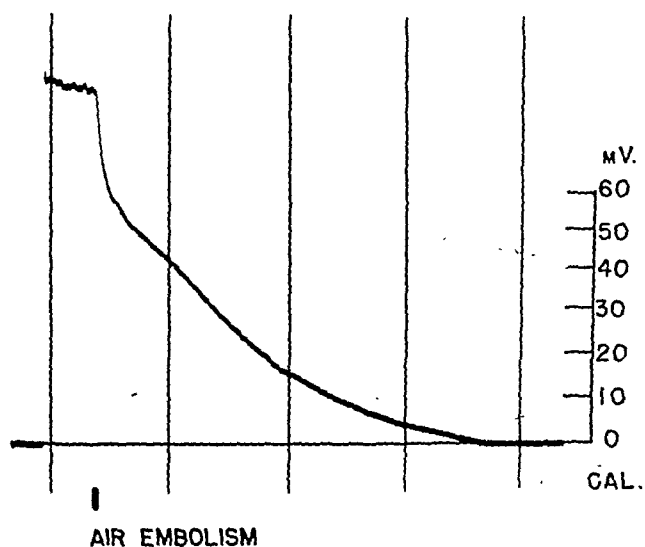


Fig. 9. Air embolism caused a reduction in potential to half the former voltage in 5 minutes. Voltage fell to zero in 30 minutes.

bly be necessary to continue the stimulus for several minutes to demonstrate results. During this time the record is made invalid by the static charge. Nevertheless, there is evidence that on some occasions femoral nerve stimulation, and central and peripheral vagal stimulation produced a reduction of potential. As with the various drugs used, stimulation at least did not increase the recorded potential difference.

Section of both vagi in a few cases caused a reduction of potential. Whether this resulted from mechanical stimulation due to the cutting, or to interruption of a previously active pathway cannot be stated. The number of observations is too limited to permit conclusions being drawn.

*The effect of putting food substances in the stomach.* Of 15 experiments in which various amounts (usually 25 cc.) of 5 per cent sucrose were put into the stomach, there was a slight increase of potential in 3, a decrease in 2, and an equivocal

effect or no change in 10. From these experiments it is concluded that at least in the anesthetized dog, no consistent or significant change in potential resulted from the presence of sucrose in the stomach.

In 2 experiments in which 10 per cent ethyl alcohol was put in the stomach, a definite and prolonged decrease of potential occurred. Vegetable oil in the form of corn oil did not modify the potentials.

*The amplitude of the gastric potentials in pyloric and fundic regions.* In every instance where the voltages at the pylorus and fundus were compared it was found that the fundic voltage was higher than the pyloric. The ratio varied between 2:1 and 4:3. A few tests performed to determine the effects of drugs in each region indicated unequivocally that the reactions to adrenalin, pilocarpine and histamine were the same in the two regions, showing only slight quantitative differences (figs. 7 and 8, A and B).

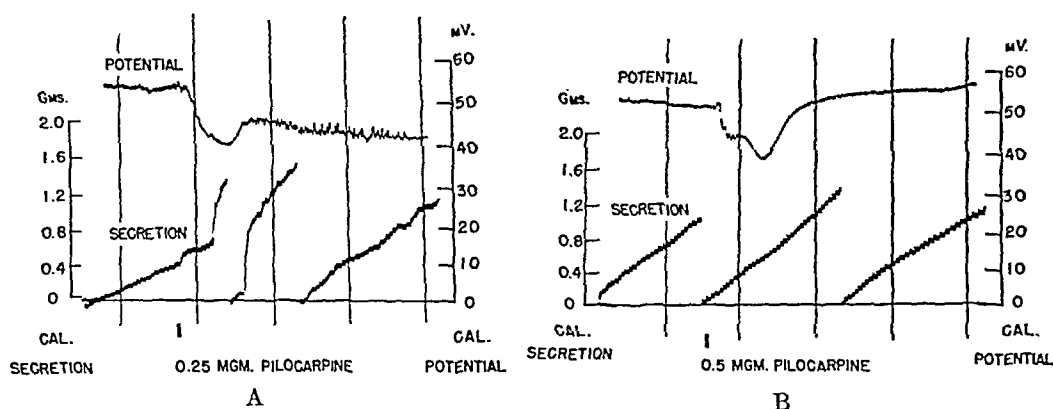


Fig. 10. A. (Left) 0.25 mgm. pilocarpine caused reduction of potential and marked increase of secretion in this dog.

B. (Right) 0.5 mgm. pilocarpine caused a reduction of potential but insignificant effect on secretion in this animal.

*Effect of death on gastric potentials.* In 38 experiments the course of the electrical record of the stomach was followed after the animal was killed by air embolism or intravenous chloroform. There appears to be no difference between the death curves resulting from chloroform and air embolism indicating that the records obtained reflect the progressive change resulting from stagnant anoxia rather than from the effect of the chloroform on the gastric cells. There was at first a precipitous fall of potential to two-thirds to one-half the former value within a minute or so. Then the curve became less steep, gradually flattening out as it approached the base line. In some instances the potential fell below the base line slightly indicating that as a terminal phenomenon the mucosal surface of the stomach may go positive with respect to the serosal surface. This is the only circumstance in which such a reversal of the normal relationship was observed. Judging from the potential curves the cells were completely dead in 10 to 30 minutes after clinical death (fig. 9).

*Relation between gastric potentials and secretory activity.* In 19 experiments attempts were made to obtain a record of the gastric secretions simultaneously with the records of potential. The results were most variable. It appears that some anesthetized animals are secreting animals and some are not. On some occasions when there was no secretion in the resting state secretion occurred in response to histamine or pilocarpine and on some occasions it did not. Occasionally when a steady secretion was manifest even in the resting state it was augmented by histamine or pilocarpine, whereas on other occasions it was not modified by these drugs. Nevertheless, whether the drugs provoked secretion from a non-secreting stomach, or whether they failed to do so; and whether they modified secretory activity from a secreting stomach or failed to do so, there was almost without exception a change in potential characteristic of these drugs and of a degree related to the doses (figs. 10 and 11). What part the

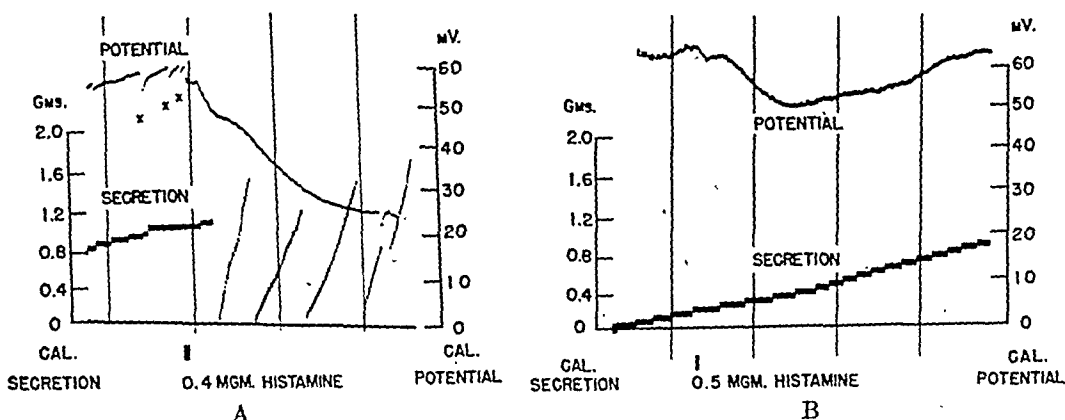


Fig. 11. A. (Left) 0.4 mgm. histamine in this dog caused marked reduction of potential and increase of secretion.

B. (Right) 0.5 mgm. histamine caused definite reduction of potential and insignificant effect on secretion in this animal.

anesthetic played in these variable results is difficult to say. When secretory activity appeared unpredictable with urethane, the anesthetic was changed to half doses of urethane and chloralose combined. The first experiment with this combination gave a good secretory response, but subsequent ones did not. Pentobarbital was then tried with results that were just as unpredictable. The greatest care was taken to exclude interference with the gastric circulation, and damage to the mucosa during the preparation, but without avail. No explanation for the unreliability of the secretory responses can be given.

**DISCUSSION.** Only a minority of previous publications concerning the potentials of the gastric mucous membrane have dealt with results obtained by continuous objective recording. Where such records have been obtained a relatively low impedance system has usually been used, with the consequent possibility of erroneous results due to the fact that the electrode resistance under such conditions may significantly affect the potentials recorded. There is

no doubt that a true picture of the changes occurring can only be obtained by continuous records, for certain changes, and the time relationships involved, are bound to suffer some loss of detail when graphs are drawn from serial visual notations.

From the experiments herein reported it is concluded, in agreement with Mond (5) and Mislowitzer and Silver (3), that the greatest potential can be recorded from the stomach when the animal is at rest. If any procedure is effective, it appears to reduce the potential. Thus the specific stimulants of gastric cells, histamine and pilocarpine, both markedly lower the potential. When the stimulant effect of pilocarpine is counteracted by atropine, the voltage commences to increase again. Contrary to the observations of Quigley et al. (6) on unanesthetized dogs, in these experiments any general disturbance appeared to lower the voltage. In humans surprise, restlessness and mental effort reduced the potential and in animals, adrenalin and stimulation (if effective at all) of both sensory nerves and the vagal efferent supply to the stomach caused a decrease. These diffuse reactions would seem to incriminate the sympatho-adrenal system as well as the specific secretory mechanisms. A change in potential resulting from activity of the sympathetic system may depend either upon a specific stimulant activity of certain cells of the stomach wall by the adrenergic system, or may reflect a non specific increase of metabolism under the stimulus of this system, or a change in the circulation through the stomach.

Although Mond (5) reported that adrenalin included in the perfusion fluid of his experiments failed to affect the gastric potentials, the evidence from these experiments shows that it has a definite and reliable effect in reducing them. Similar results were obtained by Mislowitzer and Silver (3), who also showed that histamine lowered the gastric voltages, as was found to occur in these experiments. Sarre (11) on the contrary described an increase of potential difference as occurring with histamine, whereas Quigley et al. (6) found no change. Rehm (8) reported a definite decrease. No explanation can be given for the failure of voltage changes to occur with adrenalin in the experiments of Mond or for the increased voltages noted by Sarre with histamine. It may be that the drug concentrations were inadequate. It might also be pointed out that the susceptibility of the mucous membrane to circulatory changes, especially to anoxia, make experiments involving perfusion open to some criticism. Some efforts were made to observe the potentials in the isolated stomach early in these experiments, but the mucosa showed no voltage by the time it was set up.

It is interesting to note that the duration of the effects on the gastric potentials of the various drugs employed roughly parallels the time course of their therapeutic effectiveness. But the interesting anomaly arises that although the stimulant drugs pilocarpine and histamine do not necessarily evoke external evidence of their action on the mucosa in the form of secretions, they almost invariably effect a decrease of potential. It thus appears possible that the actual formation of secretory products represents the end result of a series of actions

going on in the mucosal cells, and that at times this end result may be negligible. The electrical changes however appear to be a more fundamental phenomenon for they occur reliably whether actual secretions form or not.

It is also interesting to note as observed by Mislowitzer and Silver (3) that the pylorus was found to have a lower voltage than the fundus though in these experiments the difference in voltage between the two regions was greater than that noted by these workers. It is an unfortunate complication that there is a difference between pylorus and fundus in that it makes an interpretation of the resting value of the potentials difficult in humans, where the intragastric electrode cannot be reliably and accurately placed at will. The concept that the gastric mucosal potentials are a manifestation of basic activity of the cells could be of possible significance in the interpretation of abnormalities of gastric function in the human. Indeed in this regard Goodman (2) has attempted to differentiate various gastric lesions on the basis of the recorded potentials. But when it is realized that not only the placement of the electrodes, but a wide variety of nervous and humoral influences may affect the potentials in the same direction, the interpretation of results becomes unreliable in the present state of our knowledge. Also there is a wide individual variation in the background potential of the stomach in different individuals. Added to these physiological factors, the technical details involved, such as the avoidance of spurious polarization potentials due to the electrodes, and the avoidance of changing resistance in the leads due to gas block in the saline bridges, etc., increase the difficulties of interpretation in humans.

Nevertheless it would appear that some possible fields of usefulness may be worked out through the application of known influences on the gastric potentials. The first is the relationship between the emotional background and the lability of the potentials. It seems not unreasonable to think of the possibility of variable activity of gastric cells showing up as fluctuating voltages, even (as suggested by these experiments) independent of the amount of acid or other secretions, and of such voltage changes being a measure of the emotional stability of the subject.

The unreliability of the secretory activity, both in the resting state and in response to stimulants, compared with the reliability of the electrical changes is of particular interest. If, as is conceivably the case, the electrical phenomena are a more fundamental manifestation of cellular activity than the actual secretory responses, they might disclose evidence of disturbed function earlier and more reliably than gastric analyses. Conversely it appears that poor secretory activity may occur in dogs with no apparent abnormality of the gastric mucous membrane, and in which the electrical responses are typical. It must be recalled however that such observations were made upon anesthetized animals. It seems possible therefore that the electrical responses to specific stimulants may reflect the condition of the cells of the stomach mucosa. It is apparent from the death curves described in these experiments that the gastric mucosa is very sensitive to changes in circulation and therefore oxygenation. Abnormal electrical responsiveness might therefore be demonstrable in diseased stomachs.

As noted above, it would appear that for various reasons, interpretation of the absolute values of potential would be difficult. But since all regions of the stomach respond in a similar manner to various procedures, variations from whatever background was present might be significant.

The evidence from the death curves that an inadequate circulation may affect the gastric potentials also poses the question whether the effect of adrenalin as well as the other drugs represents a direct action on the cells, or an indirect effect due to vasomotor influences with resultant change in potential. From these experiments no conclusion can be reached on this point, except to acknowledge that anoxia apparently can reduce the potential of the gastric mucous membrane. It would indeed be interesting and possibly of practical value, if it were subsequently found that the recordable changes in gastric potential were due to circulatory activity in the stomach, for a means would thus be provided to assess this activity.

Certain results described by Quigley et al. (6) and Goodman (2) in unanesthetized animals and humans have not been confirmed in these experiments. In the human tests, drinking milk or sugar solutions led to no consistent changes. Certainly in the few human subjects observed, an increase of potential rarely occurred from the ingestion of sucrose, alcohol or corn oil. This failure seems to be explained by the fact that any disturbance, whether in the form of slight physical effort, sensory stimulation or attentiveness from a completely relaxed state modified the voltage being recorded. It therefore became impossible to determine whether the substance being taken into the stomach produced the changes noted or whether the changes were merely a result of the effort and disturbance involved in taking the substances. The successful avoidance of these complicating factors, and the development of a suitable and reliable procedure to permit an interpretation of the voltage changes occurring in the human stomach has apparently still to be worked out.

#### SUMMARY

1. A brief review of the pertinent literature concerning the electrical potential of the gastric mucosa was presented.

2. Apparatus and procedures for obtaining continuous photographic records of gastric potentials and secretions were described.

3. Limited results of experiments on a small number of human subjects, and detailed results pertaining to gastric potential and secretions in dogs were presented and discussed. These results disclosed that the resting voltage of the stomach ranges between 40 and 100 millivolts. The mucosal surface is negative with respect to the serosal surface. The voltage attains its maximum when the stomach is at rest and the animal quiescent. Interference or trauma to the stomach lowers the potential difference across the mucosa. Systemic disturbance to other parts of the body, as by painful stimulation, also lowers the voltage, as does adrenalin. These observations indicate that the voltage may be modified by the sympatho-adrenal system.

Also, the stimulant drugs pilocarpine and histamine reduced the gastric potential. The decrease caused by these drugs, especially pilocarpine, was prolonged. The reduction of voltage caused by all drugs tested roughly paralleled the time course of their therapeutic effectiveness.

4. The reduction of potential caused by pilocarpine and histamine was sometimes associated with increased gastric secretions and sometimes not. Secretory responses of the stomach to these drugs were unreliable, whereas the electrical responses almost invariably occurred.

5. Possible explanations for the results observed were presented and discussed. Some remarks were made relating to the possible diagnostic value of the gastric potentials.

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# THE INTERACTION OF PROTHROMBIN A AND B

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The hypothesis has been presented by Quick (1, 2) that prothrombin is composed of two components, which he designates as prothrombin A and prothrombin B. This hypothesis is based on the fact that a mixture of two different plasmas, each having a prolonged prothrombin time, has a prothrombin time markedly less than that of either of the component plasmas. Plasma deficient in prothrombin B but containing prothrombin A was either prepared by adsorption of component B with aluminum hydroxide, or obtained from animals poisoned with dicumarol. Plasma deficient in prothrombin A but containing prothrombin B was prepared by storing plasma for several days. The data presented by Quick have been confirmed by Oneal and Lam (3) but have been questioned by Seegers, Loomis and Vandenbelt (4).

We recently presented data showing that after hepatectomy in the dog both components of prothrombin decreased (5). At that time we indicated that the addition of an excess of either component partially compensated for a deficiency of the other. This observation led us to make a study of the prothrombin activity of mixtures containing various proportions of prothrombin A and prothrombin B. The data obtained show that there is a wide range of proportions over which there is a marked decrease in the prothrombin time of the mixture as compared to the values for the constituent plasmas.

**MATERIALS AND METHODS.** *Prothrombin A.*<sup>1</sup> Prothrombin A was either prepared by treating plasma with aluminum hydroxide or obtained by poisoning a rabbit with dicumarol. In each case the procedure described by Quick (1) was followed. These preparations had a prothrombin time of over 300 seconds, corresponding to a prothrombin concentration of less than 1 per cent of normal.

*Prothrombin B.* We were unable to prepare a plasma markedly deficient in prothrombin A but still containing prothrombin B by storing plasma. Studies of various procedures led us to the use of a high pH combined with standing at room temperature. Oxalated dog plasma was defibrinated by the addition of 1/10 its volume of a weak thrombin solution.<sup>2</sup> After removal of the clot the plasma was adjusted to pH 10.5 by the cautious addition of 2 *N* sodium hydroxide and allowed to stand at room temperature for 24-48 hours. At frequent inter-

<sup>1</sup> For the sake of brevity plasmas containing prothrombin A but deficient in prothrombin B or containing prothrombin B but deficient in prothrombin A will be called prothrombin A and B respectively. It is fully realized that such plasmas contain many other factors which are active in the coagulation mechanism.

<sup>2</sup> Thrombin-Topical, one ampoule diluted to 200 ml. with 0.15 *N* sodium chloride. We are indebted to Dr. Eugene C. Loomis of Parke, Davis and Company for generous supplies of this material.

vals small portions were returned to pH 7.4. These were then tested for prothrombin activity both alone and mixed with an equal volume of prothrombin A. At the point where the prothrombin time alone had reached 80–100 seconds, but that of the mixture had not increased, the entire lot of plasma was returned to pH 7.4 and kept frozen at  $-20^{\circ}\text{C}$  until used. If the plasma is kept at pH 10.5 for a longer period the prothrombin time will increase still further, but it fails to return to its original value on mixing the plasma with prothrombin B.

*Prothrombin determination.* Prothrombin was determined by Quick's method (6). This was modified in two respects: 0.1 ml of fibrinogen prepared as described by Jaques (7) was added to the plasma mixtures, and the thromboplastin

TABLE 1

*The prothrombin times of various mixtures of prothrombin A and prothrombin B*

COMPOSITION OF MIXTURE		PROTHROMBIN TIME			
Prothrombin A	Prothrombin B	Experiment 1	Experiment 2	Experiment 3	Experiment 4
<i>per cent</i>	<i>per cent</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
0	100	96	109	126	75
2	98	17.6	23.6	87	
4	96	14.9	19.4	74	
6	94	13.6	18.2	69	
8	92	13.2	17.6	60	
10	90	12.7	16.7	58	27.2
20	80	11.8	16.0	40	21.8
30	70	11.7			
40	60	11.3	17.0	37	19.7
50	50	11.8			
60	40	12.3	18.3	39	23.2
70	30	14.2			25.6
80	20	16.6	26.8	51	52
90	10	22.7	40	69	85
92	8	25.0	41	72	
94	6	27.3	51	76	
96	4	31.7	68	87	
98	2	42	145	112	
100	0	76	>10 min.	194	>5 min.

suspension and calcium chloride solution were mixed in equal proportions; 0.2 ml. of this mixture then was added to the plasma-fibrinogen mixture and the clotting time measured from this point.

*Prothrombin mixtures.* In studying the interaction between the two components, mixtures of the two types of plasma were made containing 0–100 per cent of prothrombin A, and, conversely, 100–0 per cent of prothrombin B. In this way a series of plasma mixtures were obtained having continually increasing concentrations of prothrombin A and correspondingly decreasing concentrations of prothrombin B.

Four different types of mixtures of prothrombin A and prothrombin B were studied. The data for each of these combinations are shown in table 1. In

experiments 1 and 2 the prothrombin B used was oxalated dog plasma treated as described. In experiment 1, the rabbit was given dicumarol only until the prothrombin time was 70–80 seconds, while in experiment 2 dicumarol treatment was continued until the prothrombin time was over 10 minutes. In experiment 3 the prothrombin A was dog plasma treated with aluminum hydroxide as described by Quick (1) while the prothrombin B was human plasma which had been stored in cotton plugged test tubes at 5°C for four months. The plasma was returned to its original volume with distilled water before using. In experiment 4 the prothrombin preparations were partially purified by adsorption on aluminum hydroxide followed by elution with phosphate buffer by the procedure

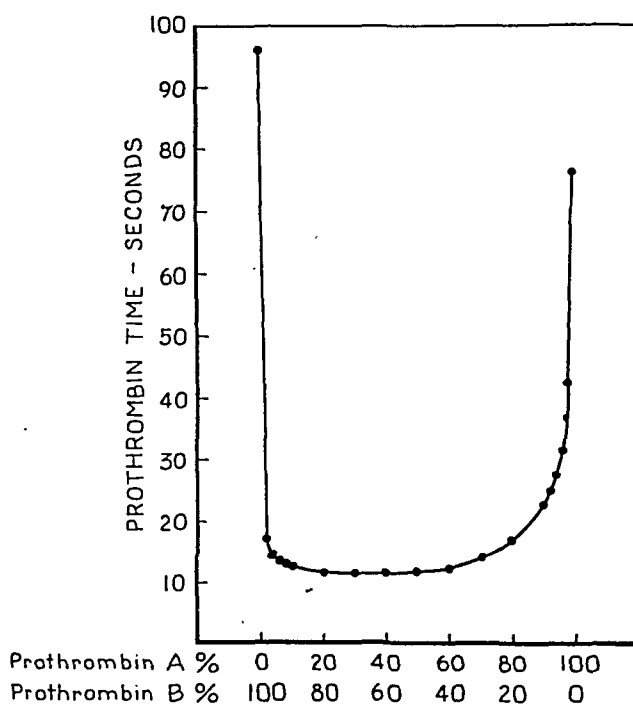


Fig. 1. The effect of varying concentrations of prothrombin A and B on prothrombin time.

described by Munro and Munro (8). The prothrombin A was prepared from plasma from a dicumarol treated rabbit and the prothrombin B from dog plasma treated as previously described.

**RESULTS.** The data obtained in the four experiments are given in table 1, and the values for experiment 1 are shown graphically in figure 1. There are two observations to be made regarding the prothrombin times obtained in all the experiments. First, as shown particularly by experiment 1, there is practically no change in prothrombin time over the range of mixtures from that containing 90 per cent prothrombin B and 10 per cent prothrombin A to that containing 40 per cent prothrombin B and 60 per cent prothrombin A. Second, the curve in which prothrombin time is plotted against concentration is not sym-

metrical. In every experiment the increase in prothrombin time appears on the one side in mixtures where the concentration of prothrombin A is above 50 per cent and that of prothrombin B below 50 per cent, while it does not appear on the other side until the concentration of prothrombin B is above 90 per cent and that of prothrombin A below 10 per cent.

DISCUSSION. The data presented here suggest that the interpretation of the prothrombin times obtained for mixtures of prothrombin A and B must be made with caution. At present it does not seem possible to put a quantitative interpretation on the prothrombin times obtained by adding a given amount of one component to a plasma deficient in that component. The fact that two plasmas, each having a prolonged prothrombin time, give a much shorter prothrombin time when mixed does not provide much information regarding the concentration of the prothrombin components in the two plasmas. Since there are a number of concentration ratios which give the same prothrombin time, the prothrombin time of such a mixture could result from the original plasmas having widely varying contents of prothrombin A or prothrombin B. It is, for example, not possible, just because a sample of plasma has a prolonged prothrombin time which returns to a normal level on the addition of prothrombin A, to state that the prothrombin time was prolonged only because the plasma is deficient in prothrombin A. On the basis of our data it is quite possible for the plasma to be markedly deficient in prothrombin A and also slightly deficient in prothrombin B.

It must again be emphasized that in the experiments discussed here we are not dealing with pure components of prothrombin. In no case can we state with certainty that the plasma preparations used contained one component to the exclusion of the other. It is obvious that a plasma having a prothrombin time of 80-100 seconds, while markedly deficient in one component, does contain a small amount of that component. On the basis of our data it is apparent that the addition of only a small amount of the deficient component can make a very marked change in the prothrombin time obtained.

The asymmetry of the curves obtained with these mixtures can probably be explained on the basis of the suggestion previously made that a prolonged prothrombin time is not necessarily conclusive evidence that the material under investigation is deficient in only one component. It is quite possible that the prothrombin B preparations we have used owe their prolonged prothrombin time to a combination of a marked deficiency of prothrombin A with a partial deficiency of prothrombin B, while the converse holds for the prothrombin A preparations. Since we are unable to state that a prothrombin A preparation having a prothrombin time of, for example, 80 seconds, has a prothrombin A content equivalent to the prothrombin B content of a prothrombin B preparation having the same prothrombin time, there is no reason to assume that the range of minimum prothrombin times would fall equally on each side of the 50 per cent mixture.

#### SUMMARY

The prothrombin time of mixtures of prothrombin A and B have been deter-

mined. It has been shown that these mixtures have a wide range over which the prothrombin time is a minimum. The curve obtained by plotting prothrombin time against concentration of either component is not symmetrical.

We wish to acknowledge the technical assistance of Miss Annabel Avery, B. A.

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# THE EFFECT OF INSULIN ON FOOD INTAKE AFTER VAGOTOMY AND SYMPATHECTOMY

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It is well known that insulin induces strong sensations of "hunger" in normal humans (1) and under appropriate conditions food intake can be increased in both man and animals by the administration of insulin (2, 3). It is furthermore well established that insulin hypoglycemia stimulates gastric peristaltic activity (4). It has been assumed that the hunger sensations and augmentation of food intake are the result of increased motor activity induced by insulin (4).

It has been demonstrated that vagotomy abolishes the gastric motor response to insulin (5). This investigation was undertaken in order to determine whether the augmentation of food intake produced by insulin would be prevented by abolishing, by means of vagotomy, the gastric motor response to insulin.

We desired an answer to this question because it would provide information of significance in the formulation of our concepts of hunger and appetite, as will be brought out in the discussion.

**METHODS.** Five mongrel dogs of both sexes were used in this study. In three of the five animals extrinsic denervation of the stomach had been accomplished by a three-stage operative procedure from 3 to 5 months prior to the beginning of the present experiment. The three stages consisted of 1, supra-diaphragmatic bilateral vagotomy; 2, right splanchnicotomy and excision of the right lumbar sympathetic chain, and 3, left splanchnicotomy and excision of the left lumbar sympathetic chain.

The animals were kept in individual cages in an air-conditioned dog room. The feeding procedure was kept as rigidly standardized as possible. The feeding was done by the same person each day, and at the same hour of the day. The food pan was allowed to remain in the cage for exactly one hour and the weight of food consumed during that time was determined and recorded.

The diet consisted of a commercial dried dog food (Pard, Swift) which was moistened with freshly prepared broth of horse meat.

Body weight was recorded once each week.

**RESULTS.** The animals used in this study had been used for previous studies on food intake and so were accustomed to the feeding procedure.

During a three week control period the average body weight and the average daily food intake remained relatively constant in both the dogs with denervated stomachs and the normal dogs (see fig. 1).

During the ensuing five week period each animal received a daily subcutaneous injection of regular insulin (Iletin, Lilly) in a dose ranging from 0.25 to 0.5

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U/kgm. one hour before the regular feeding time. It was necessary to stay within this low dosage range because the sympathectomized dogs were extremely sensitive to insulin and developed convulsions with only slightly higher doses.

Figure 1 reveals that the average daily food intake during the first week of insulin treatment was increased to 155 per cent and 148 per cent of the pre-insulin control level in the dogs with denervated and normal stomachs respectively. During the remaining four weeks of insulin treatment, the average daily food intake gradually diminished in spite of moderate increase in the insulin dosage. However, even at the end of the insulin treatment period the average daily food intake was still significantly higher than the control level.

Upon discontinuing the insulin injections the food intake rapidly returned to approximately the control level.

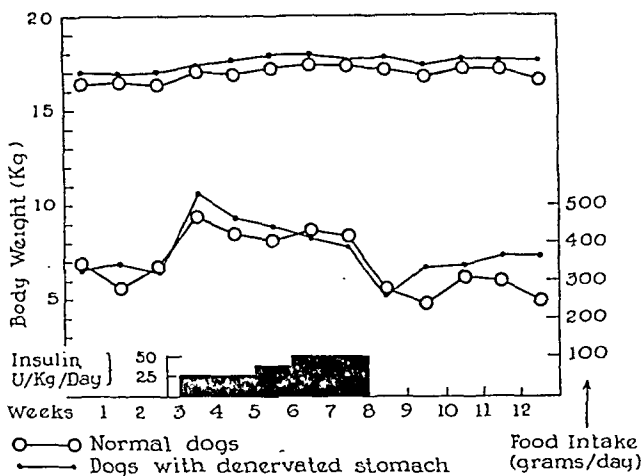


Fig. 1

The average body weight showed a slight increase during the period of augmented food intake under insulin treatment.

**DISCUSSION.** The notable finding brought out by these studies is that insulin increases the food intake in both the normal animals and in those with extrinsically denervated stomachs. If the increased food intake were due to the stimulation of gastric motility by insulin, then it would not be expected to occur after vagotomy which abolishes the gastric motor response to insulin.

How, then, may the insulin be presumed to act to cause the increase in food intake? It is reasonable to suggest that the insulin-induced hypoglycemia may act directly upon the brain to excite "hunger". Of course, the possibility that some peripheral effect, such as, for example, the effect of the vagus on the esophagus, is still responsible for the increase in "hunger" cannot be ruled out. However, if the vagal effect upon the esophagus were a factor in the phenomenon it would be expected to operate in conjunction with the vagal effect upon the stomach and duodenum. Therefore the fact that the augmentation of appetite is as great after denervation as it is before denervation can be considered as very

strong evidence favoring the view that the insulin effect on food intake is central and not peripheral in origin.

It cannot be stated whether it is the hypoglycemia per se which acts as a stimulus upon the brain, or whether the hypoglycemia acts upon another organ which in turn produces its effect upon the brain.

Theoretically there are two mechanisms by which insulin hypoglycemia could act upon the brain to produce augmentation of food taking; namely, 1, it could stimulate a hypothetical "hunger center" (a hunger center in the sense postulated by Pavlov (6)), or 2, it could act upon learned food taking patterns, i.e. "appetite", in some unknown way. The facts at hand do not permit us to make a choice between these two possibilities.

We consider our evidence to suggest the possible existence of a hunger center in the brain and we are currently engaged in further exploring this possibility.

Further confirmation of the fact that the effect of insulin on "hunger" can be independent of vagal innervation has been obtained from studies on patients who have been subjected to bilateral supradiaphragmatic vagotomy for the treatment of peptic ulcer. Injection of insulin in these patients is reported to produce strong sensations of "hunger" both before and after vagotomy (7).

After these studies had been completed it was discovered that Morgan and Morgan (3) had performed similar experiments upon rats and arrived at a similar conclusion regarding the dispensability of the vagus for the effect of insulin upon food intake.

#### SUMMARY AND CONCLUSIONS

Insulin causes augmentation of food intake both in normal dogs and in dogs with extrinsically denervated stomachs. This finding strongly suggests that the insulin hypoglycemia acts directly upon the brain to excite food taking activity.

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# THE EFFECT OF VARIOUS DEGREES OF ANOXIC ANOXIA ON WATER DISTRIBUTION IN THE BODY

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In 1928 Smith (1) reported that dogs and rats showed a definite water retention when subjected to relatively small reductions in barometric pressure (26 to 98 mm. Hg) corresponding approximately to altitudes from about 800 to 4,000 feet. He demonstrated that the disturbance of water balance of the body was accompanied by restlessness and postulated that this hydration might be the mechanism inducing the reactions on the part of many animals and some people to changing weather conditions.

Sundstroem (2) found that the water content of the liver was significantly increased in rats exposed to a barometric pressure of 300 mm. Hg (approximate altitude of 23,000 ft.) for a period of four weeks. From these findings he predicted that animals exposed to very low barometric pressures would have a higher water content in their tissues.

As far as the authors are aware no extensive studies have been reported on the effect of short periods of various degrees of anoxic anoxia on the water content of different tissues of animals.

**METHODS.** Male Wistar albino rats of 110-150 days were used. Paired animals were weighed; one was placed in a low-pressure chamber and the other (control) kept in a cage which stood adjacent to it. No food or water was permitted either animal during the course of the experiment. At the end of 3½ hours they were weighed, decapitated and autopsied.

The following barometric pressures were used: 564, 379 and 246 mm. Hg, corresponding to partial pressures of oxygen of 117, 80 and 53 mm. Hg (approximate altitudes of 8,000, 18,000 and 28,000 ft., respectively).

The water content of the following tissues was studied: liver (entire organ), adrenals (both organs), brain (cerebrum), muscle (abdominis rectus and quadratus), skin (portion weighing 1-2 grams from back and from abdomen from which hair had been removed), kidney (one entire organ).

Organs or parts thereof were weighed accurately on tared watch glasses and placed in a drying oven at 105°F. The tissues were weighed daily (starting 48 hrs. after autopsy) until two successive weighings varied by 0.5 mgm. or less. The loss of weight was considered to represent water. Average percentage water loss for test and control animals was determined and compared by means of the "t" test according to Fisher (3). A probability of 0.05 or less was considered as indicating a real difference in water content.

**RESULTS AND DISCUSSION.** The results indicate that animals subjected to various degrees of anoxic anoxia for a period of 3½ hours show no significant

TABLE 1  
*Water content of various organs during anoxic anoxia*

ORGAN	CONTROL		EXPERIMENTAL				
	No. of animals	% of water	No. of animals	% of water	Difference	"p"	"p"
Barometric pressure 564.4 mm. Hg							
Cerebrum.....	18	78.74	19	78.67	-0.07	0.56	0.6
Kidney.....	18	76.47	19	76.26	-0.21	0.84	0.4
Liver.....	17	70.69	19	70.41	-0.28	0.74	0.5
Muscle (rectus).....	18	74.62	19	73.78	-0.84	1.04	0.3
Skin.....	18	65.68	19	65.29	-0.39	0.43	0.7
Adrenals.....	18	76.86	19	77.41	+0.75	0.37	0.7
Barometric pressure 379.4 mm. Hg							
Cerebrum.....	18	78.23	20	78.25	+0.20	0.19	0.8
Kidney.....	18	76.04	20	75.86	-0.18	0.55	0.6
Liver.....	18	71.36	19	70.67	-0.70	1.46	0.15
Muscle (rectus).....	18	73.41	20	71.90	-1.5	2.54	0.01
Skin.....	17	65.73	18	64.24	-1.49	2.03	0.05
Adrenals.....	12	71.08	13	73.15	+2.07	1.24	0.20

TABLE 2  
*Barometric pressure 246.8 mm. Hg*

ORGAN	CONTROL		EXPERIMENTAL				
	No. of animals	% of water	No. of animals	% of water	Difference	"p"	"p"
Group A							
Cerebrum.....	19	78.66	18	78.78	+0.12	0.95	0.3
Kidney.....	19	76.31	20	76.55	+0.24	0.98	0.3
Liver.....	18	70.66	20	70.73	+0.07	0.21	0.8
Muscle (rectus).....	19	73.77	20	73.55	-0.22	0.238	0.8
Skin (of back).....	18	65.47	19	64.28	-1.19	1.27	0.2
Adrenals.....	17	73.25	19	77.08	+3.83	2.78	0.01
Group B							
Muscle (quadratus).....	16	76.89	16	76.12	-0.77	1.38	0.2
Muscle (rectus).....	16	73.31	16	71.72	-1.59	0.87	0.4
Skin (of back).....	16	65.90	16	65.02	-0.88	0.52	0.6
Skin (of abdomen).....	16	63.61	16	64.34	+0.73	0.24	0.8
Adrenals.....	16	77.90	16	79.31	+1.41	0.69	0.5
Group C							
Muscle (quadratus).....	13	76.46	13	77.20	+0.74	1.00	0.3
Muscle (rectus).....	13	73.61	13	73.64	+0.03	0.02	0.9
Adrenals.....	13	71.53	12	72.63	+1.10	0.67	0.5

change in the water content of several important tissues of the body. The data in tables 1 and 2 indicate that the adrenals show an increase in water content following anoxia. The results, however, are inconclusive in that the statistically significant increase observed in the weight of the adrenals in group A (table 2) could not be confirmed in groups B and C. The water content of muscles and skin following anoxia is indeterminate; in some instances the amount of water was increased and in others decreased. The remainder of the tissues studied, namely, cerebral, kidney and liver likewise showed no important change in water content following anoxia.

It has been known for some time that animals subjected to an effective degree of anoxic anoxia lose weight. Recently Stickney (4) in this laboratory has shown that rats subjected to anoxic anoxia for a period of  $3\frac{1}{2}$  hours suffer a loss of weight proportional to the simulated altitude up to 28,000 feet, that is, anoxia increases the degree of body weight loss in proportion to its severity.

In the experiments which we are currently reporting all of the animals lost a significant amount of weight following a  $3\frac{1}{2}$  hour exposure to anoxic anoxia, even at a simulated altitude of 8,000 feet. It is of interest to discuss briefly through what channels the animals lose so much weight.

It is known that anoxic anoxia produces a polyuria in unanesthetized animals; this would account for some loss of body weight. Moreover anoxic rats were observed by Stickney (4) to defecate more often than the control animals. The hyperventilation produced by anoxia, too, would cause increased moisture to be lost from the body. Swann and Collings (5) have demonstrated that the increased insensible water loss at altitude was due to the specific effect of anoxia.

Since the animals all lost a significant amount of body weight and since the various organs studied did not show a significant loss or water, we are forced to conclude that hemoconcentration took place. There is in fact considerable evidence that hemoconcentration does take place at high altitudes; some observers maintain that the polycythemia of high altitude can be explained, in a measure at least, in this way. The authors do not wish to imply that hemoconcentration is the only explanation of their findings. It is currently accepted that during dehydration the primary water loss is from the blood; this loss is in part compensated for at the expense of the extra-cellular water.

A reasonable interpretation of our findings, however, would be that the body in order to maintain its homeostatic state allowed the blood (and probably the extra-cellular spaces) to lose water rather than the tissue cells. If the anoxia were continued long enough, however, eventually the tissue cells would suffer a water loss as well and the body as a consequence would lose its homeostatic state. As previously mentioned, Sundstroem (2) found that rats following a prolonged period of anoxia showed a significant increase in water content of the liver. The inconstant results obtained in our experiments indicate that we were dealing with a threshold duration of anoxia.

#### SUMMARY

Albino rats were subjected for  $3\frac{1}{2}$  hours to the following barometric pressures: 564 mm. Hg, 379 mm. Hg and 245 mm. Hg, approximate altitudes of 8,000,

18,000 and 28,000 feet respectively. The water content of the cerebrum, kidney, liver, muscle, skin and adrenal glands was determined.

The results obtained were indeterminate. In some animals the tissues of certain organs lost a significant amount of water while in others under the same conditions this did not obtain.

At each elevation the animals invariably lost significantly more weight than the corresponding control animals.

It was possible that in order to maintain its homeostatic state the body produced a hemoconcentration in order to protect the tissue cells of the body from water loss.

We wish to express our sincere thanks to Charles Woodrow Thacker and to Robert Earle Richard for their technical assistance.

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# THE INFLUENCE OF TEST MEAL COMPOSITION ON GASTRIC EMPTYING IN MAN\*

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It has been frequently demonstrated in both animals and man that the ingestion of the individual nutrients, sugar (1-7), fat (2, 3, 5, 8-15) and protein (2, 16, 17), may exert an inhibitory effect on gastric motility. In most of the investigations the test substances have been used in doses so concentrated as to be rarely encountered in voluntary eating and their effects have not been referred to a control test meal of generally accepted composition. This does not in the least detract from the value of the observations *per se* but it does raise the question of applicability of the results to the problems of general nutrition.

The present report is a comparison of the gastric emptying sequence in 8 normal young men with five variations of a standard basic gastric test meal.

**METHODS.** Eight normal young men free from gastrointestinal abnormalities were studied during a period of seven months while maintained on a carefully standardized dietary. The gastric test meals were given at intervals of two weeks to three months.

The basic test meal consisted of 40 grams of dry oatmeal cooked in sufficient lightly salted water to make a 400 gram total after the addition of 60 grams of barium sulfate. In the special test meals, part of the oatmeal was isocalorically replaced with either sucrose, lactalbumin, butter fat or hydrogenated vegetable oil. All the test meals were of equal volume, consistency and caloric content. The composition of the various test meals and the sequence in which they were used are given in table 1. It will be seen that the several test meals provided a range, as per cent of calories, from 25 to 85 for carbohydrate, 8 to 70 for fat and 5 to 55 for protein.

The warm test meals were rapidly eaten in one to two minutes. Roentgenograms (36 inches) were taken at 5, 30, 60 and 90 minutes after the meals were eaten. After the 90 minute x-ray film, the progress of the meals was followed fluoroscopically at 15 minute intervals. The final emptying was confirmed by an x-ray picture. The x-rays and fluoroscopy were done with the subjects in the standing position. Between observations the subjects were at seated rest.

The gastric shadows were traced onto paper from the developed x-ray films and the areas measured with a planimeter. All gastric areas were expressed as per cent of the area at five minutes after the meals were eaten.

**RESULTS.** The results for the various test meals are summarized in tables 2 and 3.

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The first plain oatmeal test meal was used as the basis for comparison of the other test meals. However, the second plain oatmeal test or the average of the first and second oatmeal tests could be used as the basis without influencing the statistical results to any appreciable extent.

TABLE 1

*Composition of the test meals and sequence in which they were used*

PER CENT OF CALORIES AS	1ST PLAIN OATMEAL	OATMEAL PLUS SUCROSE	OATMEAL PLUS LACTAL- BUMIN	OATMEAL PLUS BUTTER FAT	OATMEAL PLUS VEGETABLE OIL	2ND PLAIN OATMEAL
Carbohydrate.....	69	85	36	25	25	69
Fat.....	17	8	9	70	70	17
Protein.....	14	7	55	5	5	14

TABLE 2

*The average per cent of each test meal emptied from the stomach at 30, 60 and 90 minutes, and the average final emptying times, the ranges and the standard deviations of the mean final emptying times in minutes*

TEST MEAL	% EMPTIED AT			FINAL EMPTYING TIME		
	30 min.	60 min.	90 min.	Mean	S.D.	Range
1st plain oatmeal.....	48	79	88	150	±50	105-270
Oatmeal-sucrose.....	54	74	84	139	±19	120-180
Oatmeal-lactalbumin.....	42	68	81	190	±58	120-225
Oatmeal-butter fat.....	45	77	84	169	±47	120-270
Oatmeal-vegetable oil.....	44	67	83	150	±29	105-180
2nd plain oatmeal.....	43	69	83	158	±41	135-255

TABLE 3

*The average time in minutes required for the evacuation of various percentages of the test meals*

TEST MEAL	40%	50%	60%	70%	80%	90%	100%
Plain oatmeal.....	25	33	42	52	65	102	150
Oatmeal-sucrose.....	22	28	39	54	79	108	139
Oatmeal-lactalbumin.....	28	39	51	65	87	135	190
Oatmeal-butter fat.....	26	35	44	54	75	120	169
Oatmeal-vegetable oil.....	27	38	51	66	85	116	150
Plain oatmeal.....	27	37	50	61	82	117	158

The mean emptying times for the various test meals were all well within the range of normal variation except for the oatmeal-lactalbumin meal which required 40 minutes longer to empty than did the first plain oatmeal test meal. The difference is, however, not statistically significant. A comparison of the per cent of the test meal emptied at 30, 60 and 90 minutes also indicates that

increasing the per cent of calories from carbohydrate, fat or protein in the test meal had no significant effect on the course of gastric emptying.

The high protein test meal was the slowest to empty while the high carbohydrate was the fastest (190 and 139 min., respectively). The difference of 51 minutes in the mean emptying time between the two test meals was statistically significant— $t = 2.39$  with  $t = 2.36$  for the 5 per cent level of significance. The slightly greater gastric motility with the high carbohydrate test meal is apparent throughout the entire period of the test.

Using the first plain oatmeal test as the basis for comparison, the per cent of subjects in whom the final emptying time on the various test meals differed by more than  $\pm 15$  minutes is given in table 4. In none of the variations of test meal composition was the distribution significantly changed except with the high protein where 6 of the 8 subjects had prolonged emptying times of 30 minutes or more.

A comparison of the two plain oatmeal test meals (the first and last tests in the series) indicate that with a group of 8 men there is a good repeatability of

TABLE 4

*Per cent of subjects who showed a  $\pm 15$  minute difference in final emptying time using the first plain oatmeal test meal as a basis*

	OATMEAL PLUS SUCROSE	OATMEAL PLUS LACTALBUMIN	OATMEAL PLUS BUTTER FAT	OATMEAL PLUS VEGETABLE OIL	2ND PLAIN OATMEAL
Longer.....	12.5	75.0	37.5	37.5	25.0
No change.....	62.5	12.5	62.5	37.5	50.0
Shorter.....	25.0	12.5	0	25.0	25.0

the average results (tables 2 and 3). The mean emptying times differed by only 8 minutes and are comparable to those observed in this Laboratory on other occasions (18). This does not mean, however, that for any one individual gastric motility will be substantially the same from day to day. In our group of subjects who were living under a rigorously controlled regimen of activity and diet, only 4 of the 8 had final emptying times with the second plain oatmeal test meal that were within  $\pm 15$  minutes of the emptying times with the first plain oatmeal test (table 4). In two subjects the emptying times differed by 30 minutes and in two subjects the differences were 90 and 135 minutes. The rank correlation between the emptying times of the two tests was  $-0.22$ .

The rather large inter-individual and intra-individual variability observed even when experimental conditions were rigorously controlled demonstrates the necessity for extreme caution in the interpretation of results from studies based on few subjects and incompletely controlled conditions. A comparison of the responses of one individual to various test meals which differ greatly in size, composition and consistency may lead to very erroneous conclusions.

Studies of gastric motility that depend on observations until the test meal has been completely evacuated from the stomach are very time consuming for both

the subjects and the observer. The possibility of predicting the final emptying time from the motility rate during the earlier phases of emptying was investigated. For this the results from 8 men on the 6 test meals were grouped together to give 48 individual man-tests. Product-moment correlations were calculated for the per cent of the test meal left in the stomach at 30, 60 and 90 minutes, and the final emptying time. The correlations of the final emptying times with the emptying during the first 30, 60 and 90 minutes were  $+0.492$ ,  $+0.597$  and  $+0.664$ , respectively. It is apparent that in individual cases it would be impossible to predict satisfactorily the final emptying time from the motility rate during the first 90 minutes of gastric emptying. Conversely, the final emptying time does not give an accurate picture of the average rate of gastric evacuation at any time prior to final emptying.

DISCUSSION. In an ordinary mixed diet the total daily calories are derived about 30-35 per cent from fats, 15 per cent from proteins and 60-65 per cent from carbohydrates. Although the percentages vary with food habits and availability of food items, food as eaten is almost without exception a mixture of fats, carbohydrates and proteins. The practical consideration in nutrition is whether altering the percentages within normal or reasonable limits will profoundly change gastric activity. From the present experiments it appears that such is not the case in healthy young men. It must be recognized, however, that the test meals used, although they are quite comparable to the test meals used clinically, are calorically more dilute than the food items in ordinary meals. It should be noted, however, that most persons take additional fluids with their meals. In any case there is no specific evidence at present that the comparative response to test meal composition in calorically more concentrated test meals would differ significantly from the responses observed in this experiment.

The suggestion has been made that candies should not be eaten before meals because they depress gastric secretion and delay evacuation (1). There is ample evidence that a concentrated sugar solution is evacuated from the stomach more slowly than is a dilute sugar solution or pure water (4, 5, 6). The candy eaten before a meal would, however, soon be thoroughly mixed with the food after the meal was eaten. The concentration of sugar in the final mixture would undoubtedly be sufficiently low to have little or no effect on gastric activity.

It is generally conceded that pure fats or fats in high concentrations have a depressant effect on gastric activity (11, 12, 14, 15). It has, however, been shown that olive oil in the usual clinical doses has no effect on gastric acidity or motility when given with a fractional test meal (19). The dilution of the olive oil by the test meal produced a final fat concentration more nearly that present in ordinary diets. It appears likely that butterfat, vegetable oil, olive oil and probably most of the other edible fats and oils do not appreciably inhibit gastric activity when diluted with other food items as is the case in normal voluntary eating habits.

The large intra-individual and inter-individual variability in response to a standard test meal even among a group of trained normal subjects indicates that

factors other than test meal composition are important in determining gastric activity. The size of the test meals (20), physical activity during or preceding the test (21) and the emotional state of the subject (22) exert considerable influence on gastric motility. In the present experiments both size of the meals and preceding physical activity were standardized. Efforts were made to avoid emotional variations and the residence of the subjects in the laboratory was helpful in gaining assurance on this point. It must be admitted, however, that even under favorable conditions emotional constancy cannot be guaranteed.

#### SUMMARY

1. The influence of test meal composition on gastric motility was observed in 8 normal young men.

2. In the variations of the basic oatmeal test meal part of the oatmeal was isocalorically replaced by fats, protein or carbohydrate.

3. The results indicate that within nutritionally reasonable limits increasing the proportion of the calories in the test meal from fat, protein or carbohydrate did not significantly alter gastric motility.

4. The rate of gastric emptying during the first 90 minutes of the emptying phase cannot be used as a reliable index to predict the final gastric emptying time in individual cases.

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EVIDENCE, FROM CROSSTRANSFUSION EXPERIMENTS, THAT  
NO TOXIC FACTOR IS PRESENT IN ISCHEMIC COMPRESSION  
SHOCK CAPABLE OF INDUCING A SHOCK STATE IN NORMAL  
DOGS<sup>1,2</sup>

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In a recent paper (1) it was reported that dogs, the hind legs of which were traumatized by being subjected to a six-hour period of ischemic compression, died within 3 to 20 hours after release of the compression in what appeared to be a state of shock. The edema in the traumatized legs was apparently significantly less than the volume of blood which would have had to be removed to cause a similar course of events. In this paper we have investigated the possibility that some toxic factor released from the traumatized tissues was responsible for the shock-like course in the traumatized (shock) dogs by recording the mean arterial pressure and the length of survival of normal test dogs cross-transfused with such traumatized dogs.

**METHODS.** The hind legs of dogs were traumatized by tightly wrapping each leg with a 10 foot strip of amber tubing of  $\frac{3}{8}$  inch internal bore in a continuous spiral from the ankle to groin. The tubing was removed after 6 hours of such ischemic compression. Dogs subjected to such manipulation will hereafter be designated traumatized (shock) dogs.

Two procedures were used for attempting to detect the presence of a toxic factor. In a first the traumatized legs were isolated and perfused with a reservoir-pump-lung system and the blood in the reservoir exchanged with the blood of a test dog. These experiments were controlled by perfusing non-traumatized legs in a similar manner, and exchanging the perfusate with the blood of a test dog.

In the second set of experiments the blood of the traumatized dog was continuously exchanged with that of a test dog. In order to provide for a rapid rate of exchange and at the same time to be certain that the volume of blood supplied by the test dog exactly equalled that which it received from the traumatized dog, the crosstransfusion was carried out through a stromuhr similar to the Ludwig model. As shown in figure 1, blood from the traumatized dog entered the bottom of the right hand tube from either the carotid artery or the inferior vena cava and at the same time displaced an exactly equal quantity of blood from the left hand tube into its jugular vein. When the right hand

<sup>1</sup> Supported in part by a grant from the Commonwealth Fund.

<sup>2</sup> A preliminary report of this work appeared in the Federation Proceedings 4: 26, 1945.

tube was full the stopcocks were reversed. Blood then entered the left hand tube from the carotid artery of the test dog while an exactly equal quantity of blood (which came from the traumatized dog) entered the jugular vein of the test dog. The stopcocks were rotated by an electric motor controlled by electronic relays which were actuated by contact of the blood with the electrodes at the top of the vertical tubes. The stroke volume of the apparatus was adjusted usually to approximately 50 ml. The rate of exchange was indicated by a signal magnet which recorded each reversal of the stopcocks. The details of the apparatus and electrical circuits are described elsewhere (2). All parts of the apparatus and its connections, which came in contact with

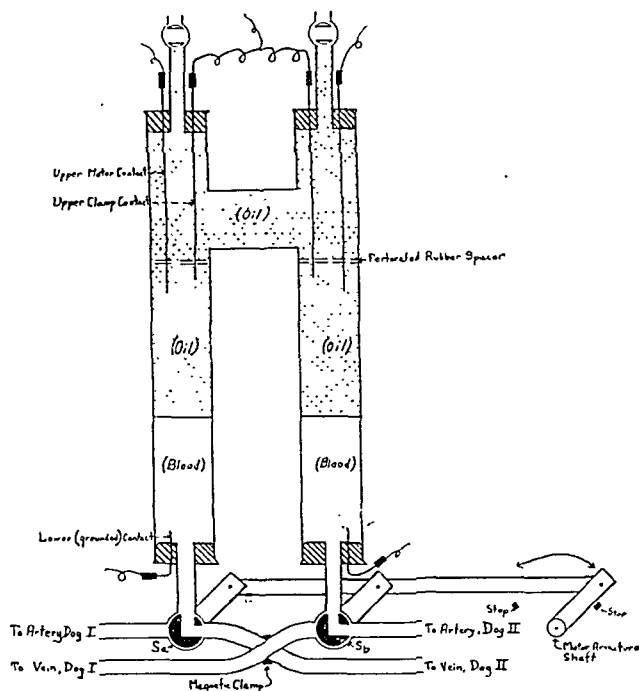


Fig. 1. Stromuhr apparatus used for continuous cross-transfusion. For explanation see text p. 112.

the blood were autoclaved immediately prior to each experiment and were thoroughly cleaned at the end of each experiment with soap and water. The test dogs supplied blood to the stromuhr by way of a carotid artery and received blood from the stromuhr into the jugular vein. Two types of connections were used for the traumatized dogs. In the first, blood was supplied to the stromuhr by way of a carotid artery (table 1A). In the second, the inferior vena cava, approached by an extraperitoneal incision in the right lumbar region was cannulated and the blood led to the stromuhr (table 1D). It was felt that, in this last group, most of the blood returning from the lower portion of the body, as well as that from the traumatized legs, passed through the test dog before it returned to the traumatized dog. All traumatized dogs received the blood from the stromuhr into their jugular veins.

Food was withheld for the preceding 20 hours but water was allowed up to the start of the experiment. The mean arterial pressures of both dogs were

TABLE 1

EXPT. NO.	TEST DOG DIED, HRS. AFTER ZERO TIME	DATA ON CROSSTRANS- FUSION AFTER ZERO TIME		BODY WEIGHT		BLOOD GIVEN TO TRAUMATIZED DOG	TEST DOG BLEED	AVERAGE RECTAL TEMPERA- TURE		AVERAGE ROOM TEMPERATURE	CROSS- TRANSFU- SION REACTION		MEAN ARTERIAL PRESSURE		DATE
		Duration	Vol. exchanged	Traumatized dog	Test dog			Traumatized dog	Test dog		Traumatized dog	Test dog	Zero time	One or more hrs. after end of cross- transfusion	
A. Test dogs crosstransfused with traumatized dogs—blood from carotid arteries of traumatized dogs															
		hrs.	liters	kgm.	kgm.	ml./ kgm.	ml./ kgm.	°C.	°C.	°C.			mm. Hg	mm. Hg	
KH11	surv.	4.0	20.0	10.6	11.8	0	0	38.5	38.5	24.0	0	0	150	140	25 Apr.
KH15	surv.	4.8	35.4	12.1	11.2	24.6	20.0	39.5	36.5	22.0	0	0	95	110	9 May
KH20	surv.	2.7	6.6	18.7	10.8	42.0	26.0	40.0	40.0		0	0	130	135	12 July
A28	17	4.0	21.0	14.0	9.8	26.7	0	36.5	37.0	23.0	+	+	140	100	15 Oct.
A29	19	4.6	24.0	10.9	9.6	26.6	0	37.5	36.5	27.0	0	+	100	110	24 Oct.
A31	27	4.5	25.8	14.2	5.6	24.2	0	40.0	38.0	29.0	+	+	140	115	7 Nov.
B. Controls for A28, 29, 31—test dog crosstransfused with test dog															
A30T <sub>1</sub>	surv.	5.1	27.7		11.1			37.4	29.0		+		100	105	29 Oct.
A30T <sub>2</sub>	surv.	5.1	27.7		8.6			38.7	29.0		+		80	95	29 Oct.
A32T <sub>1</sub>	12	4.1	10.0		7.9			34.3	25.6		+		60	75	12 Nov.
A32T <sub>2</sub>	surv.	4.1	10.0		8.6			35.4	25.6		+		80	115	12 Nov.
A34T <sub>1</sub>	15	4.4	13.8		6.8			35.2	27.3		0		75	105	19 Nov.
A34T <sub>2</sub>	surv.	4.4	13.8		6.7			36.7	27.3		+		120	100	19 Nov.
C. Controls for A28, 29, 31—autotransfused dogs															
A17	3.9	3.9	19.3		7.4			36.1	25.1		0		95	0	27 Aug.
A18	7.0	4.5	18.9		6.8			36.8	27.5		0		100	100	29 Aug.
A19	14.2	4.5	22.2		8.6			36.2	30.0		+		100	85	31 Aug.
A24	surv.	5.5	33.0		8.0			36.1	27.5		+		100	120	19 Sept.
A25	surv.	4.4	25.0		8.6			36.7	27.3		0		100	120	24 Sept.
A26	11.5	4.5	28.9		8.5			35.5	24.1		+		140	115	3 Oct.
A27	surv.	4.7	50.1		8.3			38.8	26.9		0		125	105	11 Oct.
D. Test dogs crosstransfused with traumatized dog's blood from inferior vena cava of traumatized dogs															
KH12	surv.	2.8	1.6	12.0	11.5	0	0	36.0	38.0	25.0	0	0	110	115	28 Apr.
KH13	surv.	1.2	1.8	14.5	13.0	0	0	41.0	40.5	27.0	0	0	160	155	2 May
KH14	surv.	4.7	7.4	12.4	10.4	52.0	0	37.0	36.0	23.5	0	0	100	115	5 May
KH21	surv.	3.8	11.3	16.3	11.0	38.5	23.5	37.5	37.5		0	+	110	155	14 July
KH32	surv.	2.2	2.2	13.9	12.8	39.0	6.0	40.5	39.0		+	+	60	110	25 June
A33	51.0	3.9	18.3	13.0	5.3	83.6	0	36.4	33.3	24.5	+	0	80	120	14 Nov.

surv. = survived indefinitely.

+

0 = no crosstransfusion reaction.

recorded with Hg manometers throughout the period of crosstransfusion and for 1 to 2 hours thereafter. The cannulas were then removed from surviving

animals, the wounds sutured and the animals placed in cages. Water and food were again allowed beginning 24 hours after the start of the experiment.

In experiments KH15, KH20 and KH21, the test dogs were bled 20, 26 and 23.5 ml./kgm. of body weight, in order to render them more susceptible to the hypothetical toxic factor. The traumatized dogs were usually heavier than the test dogs, and 9 of the former were transfused 25 to 84 ml./kgm. of blood from a donor dog in order to increase their survival and therefore presumably the amount of the toxic factor which might be made available to the test dog. The stromuhr and tubes were usually filled with blood (approximately 150 ml.) but in a few experiments saline was used. In the latter case each dog was transfused with 75 ml. of donor blood shortly after starting the crosstransfusion.

The red cells of the test dog and of the traumatized dog were crossmatched against each other's plasma and the red cells of the donor dog were matched against the plasma of both the traumatized and the test dogs. Despite the crossmatching and care in cleaning the apparatus reactions, with decline of mean arterial pressure, were seen in 13 animals.<sup>3</sup> In these the crosstransfusion was temporarily stopped until the mean arterial pressure had returned to control levels. This usually occurred within 15 to 45 minutes. In order to distinguish between cross-transfusion reactions, and the effects of any toxic factor which might appear in the circulation as a result of the trauma, the compression tubes were not released until it was certain that no reaction was occurring; or, if such did happen, until the mean arterial pressure of both dogs had returned to control levels.

Zero time in the table and charts is the moment of release of the compression of the legs of the traumatized dogs. In control experiments it is one-half hour after start of the crosstransfusion, or, in those experiments in which a reaction occurred, the moment when the crosstransfusion was started again after return of the mean arterial pressure to control levels. In all but 3 experiments the cross-transfusion was continued until the traumatized dog died.

Just before starting the crosstransfusion each dog was given 3.0 mgm./kgm. of heparin<sup>4</sup> intra-arterially. An additional 1.0 mgm./kgm. was given 30 minutes and 1 hour later, and repeated at hourly intervals thereafter as long as the crosstransfusion was continued. All dogs were anesthetized with an initial subcutaneous injection of approximately 2.0 mgm./kgm. of morphine, followed in about 30 minutes with 20 mgm./kgm. of sodium pentobarbital, intravenously.<sup>5</sup> Additional sodium pentobarbital was given as necessary to maintain anesthesia until death or until the animal had been placed in the recovery cage. During the application of the compression tubes additional anesthetic was usually required.

<sup>3</sup> A detailed report of the nature of the crosstransfusion reactions is in preparation.

<sup>4</sup> The heparin used in these experiments was liquaemin which was kindly supplied by Roche-Organon, Inc., Nutley, N. J.

<sup>5</sup> The sodium pentobarbital was supplied through the courtesy of Premo Pharmaceutical Labs., Inc., New York, N. Y.

RESULTS. I. *Perfusion of isolated legs.* Traumatized legs were artificially perfused and the perfusate exchanged with the blood of test dogs in 5 experiments. Three control experiments were performed, in which non-traumatized legs were perfused in a similar manner and the blood exchanged with test dogs. Three of the former test dogs died in 3.7, 5.3 and 9.8 hours, and two of the latter died in 1.7 and 4.8 hours. On the basis of these results it was concluded that the method of perfusion apparently caused some change in the blood which made it toxic to the test dogs. This procedure was therefore abandoned.

II. *Crosstransfusion between traumatized dogs and normal test dogs. Blood obtained from carotid artery of traumatized dogs.* Six such experiments were carried out. The results are summarized in table 1A. The test animals in the first three survived indefinitely and no controls were run. Figure 2 reproduces the results in KH15 which is considered to be typical of this group.

Some months later three more similar experiments were performed (A-28, A-29 and A-31, table 1A). In these the mean arterial pressures of the test dogs remained within normal limits throughout the period of observation as had been the case with those of the above group which had survived, but the dogs died. Three control experiments were therefore performed by cross-transfusing pairs of normal dogs. The results are summarized in table 1B. As a further control 7 dogs were autotransfused by connecting one pair of the tubes of the stromuhr to the carotid artery and jugular vein and the other pair to the femoral artery and vein of the same dog (table 1C). Tables 1B and 1C show that while the percentage survival was greater the hours of survival and the level of the mean arterial pressure of those dogs that died in two control groups were no better than those of the test animals crosstransfused with traumatized dogs.

III. *Crosstransfusion between traumatized dogs and normal test dogs. Blood obtained from inferior vena cava of traumatized dogs.* In the above experiments the total volume of crosstransfusion equalled 0.35 to 2.9 times the body weight of the traumatized dog, and amounted to 6.6 to 35.4 L., nevertheless the maximum rate of exchange was equal to only 1/10th to 1/30th the probable cardiac output of the traumatized dog. We felt it was possible, therefore, that if the hypothetical toxic factor originated in the traumatized legs and was possibly fixed by the tissues of the traumatized dog, too small an amount of it might be transferred to the circulation of the test animal. We, therefore, cannulated the inferior vena cava of a series of traumatized dogs and arranged to pass this blood through the stromuhr to the test animal. As a result of this procedure most of the blood returning from the traumatized legs passed through the test dog before returning to the traumatized dog. The results of this study are presented in table 1D. Since all the test animals survived 50 hours or indefinitely, no controls were necessary. The traumatized dogs in this group died in about the same length of time as those described in section II above.

IV. *Hematocrits and rectal temperatures.* Because of the influence of the environmental and body temperatures on the survival of traumatized dogs (3-5) an effort was made to keep the laboratory at a reasonably consistent

temperature. However, our constant temperature room was not large enough to accommodate these experiments and therefore considerable variability was experienced ( $22^{\circ}$ – $30^{\circ}\text{C}$ ). Slightly better control of rectal temperature was obtained by warming the dogs with radiant heat lamps. As shown in figures 2 and 3, the rectal temperatures of each pair of crosstransfused dogs tended to become identical during the period of crosstransfusion.

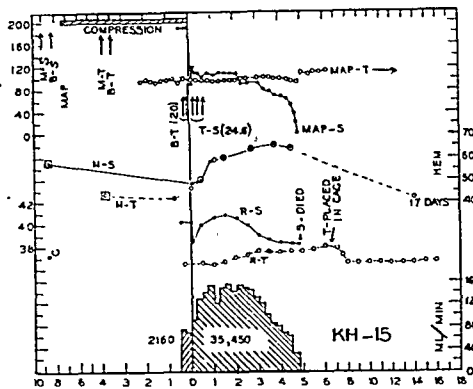


Fig. 2

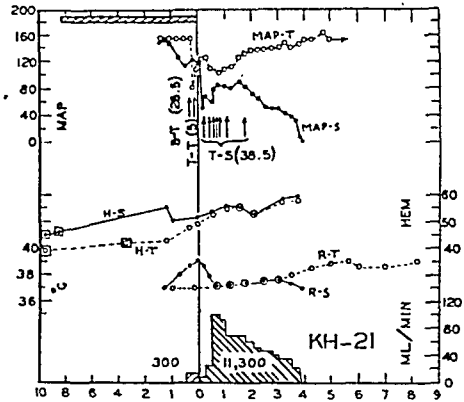


Fig. 3

Fig. 2. Results of crosstransfusion between a traumatized (shock) dog and a normal test dog, blood obtained from carotid artery of traumatized dog. *M-S* and *B-S*, initial injection of morphine and of sodium pentobarbital respectively into traumatized (shock) dog. *M-T* and *B-T* corresponding injections into test dog. Cross hatched bar in the upper left corner (compression)—interval of compression trauma of the hind legs of traumatized dog. End of compression trauma coincides with heavy vertical line intersecting zero time. Open circles—mean arterial pressure of test dog (*MAP-T*); solid circles—mean arterial pressure of traumatized dog (*MAP-S*)—scale for *MAP* at left margin. Two arrows following *B-T* (20) indicate when test dog was bled total of 20 ml/kgm. of body weight. Three arrows preceding *T-S* (24.6) indicate when traumatized dog was transfused total of 24.6 ml/kgm. of body weight of blood. *H-S*—hematocrit of traumatized dog, *H-T*—hematocrit of test dog—scale at right margin (*HEM*). Symbols surrounded by squares are hematocrits taken before administration of barbiturate anesthetic. Symbol labelled 17 days is hematocrit reading on test dog on 17th day after crosstransfusion. *R-S*—rectal temperature of traumatized dog, *R-T*—rectal temperature of test dog—scale at left margin— $^{\circ}\text{C}$ . Cross-hatched areas at bottom indicate rate of crosstransfusion in each direction in ml./min.—scale at right hand margin. Figures 2160 and 35,450 indicate total volumes of blood exchanged before and after zero time in milliliters. Scale at bottom—time in hours.

Fig. 3. Results of crosstransfusion between a traumatized dog and a normal test dog, blood obtained from inferior vena cava of traumatized dog. Symbols—same as figure 2. In addition—*T-T* (5) indicates when 5 ml/kgm. of blood was returned to the test dog.

As shown in figures 2 and 3, the hematocrits showed the characteristic initial drop following anesthetization with the barbiturate (6). That of the traumatized dog rose rapidly after release of the compression, and as a result of the crosstransfusion the hematocrit of the test dog also rose. Apparently increasing hemoconcentration reflects the loss of plasma into the traumatized tissues but does not, itself, act as a causative agent in the production of the shock state.

V. *Autopsy findings.* Autopsies were performed shortly after death on all dogs that died within 24 hours, the organs being examined grossly only and the liver, spleen, kidneys, heart and lungs weighed. In general no significant differences were noted in the organ weights between any of the groups. The 2 test animals that died as a result of exchange transfusion with the pump-perfused, traumatized legs showed only slight subendocardial hemorrhages, and slight (one plus) hyperemia of the duodenal mucosa. Similar findings were noted in the controls.

Two of the three dogs that died within 24 hours after crosstransfusion with traumatized dogs (table 1A) showed 2 plus and 4 plus hyperemia of the mucosa of the duodenum and upper small intestine. The lungs of the 2 control cross-transfusion dogs (table 1B) were deeply colored and weighed slightly more than normal. One had a normal gastro-intestinal mucosa and the other showed 4 plus hyperemia involving the entire gut with free blood in the lumen. The lungs of two of the autotransfused dogs were more deeply colored and heavier than normal and the intestinal mucosa of one of these showed 3 plus hyperemia of the duodenal and upper intestinal mucosa.<sup>6</sup>

The 12 traumatized dogs (tables 1A and 1D) showed occasional subendocardial hemorrhages in the hearts especially on the mitral valve, and in the left ventricle. All but 2 showed 2 plus to 4 plus hyperemia of the duodenal and upper intestinal mucosa with free blood in the lumen.

DISCUSSION. The earlier literature on shock, including that on the operation of toxic factors has been covered in several reviews (7-10). As these indicate, many investigators have found sufficient local loss of fluid in the traumatized and immediately adjacent tissues to initiate the shock cycle. Also more recently Aub et al. (11, 12) concluded that all the changes observed in extracellular fluid space and in sensitivity to fluid loss can be accounted for on the basis of the edema in the locally traumatized area. On the basis of the data available comparing local vs. systemic fluid loss up to 1942, however, one of us (10) concluded that these results "do not rule out the possibility of humoral or nervous factors." Furthermore, in a later publication—1944 (1)—it seemed to be demonstrated that "accumulation of fluid in the traumatized legs is an important factor in the induction of the shock state, but in many of the experiments the volume of edema appears to be insufficient to explain the death." Canzanelli, Guild, and Rapport subsequently arrived at a similar conclusion. (13).

Numerous investigators have prepared extracts from normal and traumatized tissue and injected them into test animals. Roome and Wilson (14) prepared an extract of muscle with a hydraulic press which when injected into a test dog, caused temporary decline of arterial pressure. Pen, Campbell and Manery (15) recorded toxic effects in test animals upon injection of alcoholic extracts of muscle, but obtained similar results with both normal and traumatized muscle.

<sup>6</sup> One plus hyperemia is mucosa slightly redder than normal, two plus is definitely red mucosa, three plus is velvety purple in discrete areas, four plus is velvety purple almost uniformly distributed over the mucosa.

The toxic substance, was identified in the ash of the extract as potassium. H. N. Green and Bielschowsky isolated a substance from muscle which they believe to be a pyrophosphate. Injection of this into a test animal caused a prolonged fall in arterial pressure. They believe this substance to be released into the circulation as a result of muscle ischemia (16-19). Bollman and Flock (20) found that adenosine triphosphate almost disappears from muscle after three hours of ischemia while phosphocreatine is almost completely hydrolyzed in one hour. They also observed that these substances are regenerated in the muscle if the circulation is restored within three hours and fatal shock does not occur. Release of occlusion after more than  $3\frac{1}{2}$  hours causes no regeneration of these substances and considerable inorganic phosphate is washed from the injured muscle into the blood in association with the development of fatal shock. Shorr, Zweifach and Furchgott (21) claim that a vasodepressor substance is produced in muscle slices incubated anaerobically, which induces capillary reactions similar to those seen in traumatic shock. Muirhead and Hill (22) obtained a shock-like state upon intraperitoneal implantations of reconstituted desiccated muscle, thus in essence confirming the work of Moon (7). Most of these studies thus suggest that toxic or vasodepressive substances may be obtained from injured or ischemic muscle. While such substances may play a contributory rôle, it remains to be demonstrated that in physiologically available amounts they could initiate shock in an otherwise intact animal. Progressive increases in glucose, creatine, amino acid, pyruvic acid, lactic acid and potassium have been found in the blood but with the exception of the last, none of these have been implicated as playing causative rôles (15, 23).

Phemister (24) collected small amounts of the blood draining from the vein of traumatized extremities and injected them into test animals without producing a reaction. Similar results were obtained by Smith (25), Parsons and Phemister (26), O'Shaughnessy and Slome (27), Dragstedt and Mead (28), and Selye and Dosne (29). Assuming that the toxic factor might be present in low concentration these negative results may be criticized on the basis of the small volume of blood transfused to the test animal relative to the volume of blood which would pass through the traumatized extremities under normal circumstances. Furthermore, the added volume of blood in the test animal might have masked any toxic effect. Kendrick, Essex and Helmholtz (30) perfused traumatized and normal legs artificially and obtained reactions upon injection of the perfusion fluid from the former into test animals. The results were inconclusive, however, as similar reactions were also obtained with the perfusate from normal extremities.

Aub et al. (31-36) obtained shock-like reactions in test animals upon injection of the serum which oozed from ischemic muscles after restoration of the circulation to the traumatized extremity. However, they considered the toxic product to be the result of bacterial contamination rather than of ischemic tissue necrosis. Ricca, Fink and Warren (38), and Freed, Kruger and Prinzmetal (37) arrived at similar conclusions, based on the protection obtained from the use of sulpham compounds locally or systemically in the traumatized dogs.

Haist and Hamilton (39), and Shipley, Meyer and McShan (40) found that if tourniquets were applied to the hind legs of rats for 4 or more hours, 100 per cent of the animals died in 24 hours. If, however, within 1 to 6 hours tourniquets were reapplied for 24 hours most of the animals survived. Blood glucose, pentose, lactic and pyruvic acids and plasma inorganic phosphate and amino acid nitrogen were found to increase after release of the tourniquets. These progressive changes were prevented if the tourniquets were reapplied within 6 hours (41). These results suggest that the reapplication may have stopped the entrance of toxic material from the traumatized legs into the circulation. H. D. Green and Bergeron (3), Bobb (5), and Lipton, Denison and Green (4) observed that placing the whole animal, the traumatized legs only, or the body only in a cool environment increased the hours of survival as compared to experiments conducted in a warmer environment, and allowed many dogs to survive indefinitely, although the edema in the traumatized legs was but little less in the former than in the latter. It was suggested that the cooler environment may have decreased the rate of production of, and/or, the susceptibility to a toxic factor.

McIver and Haggart (42) connected an artery and vein of a test animal to the abdominal aorta and inferior vena cava supplying the traumatized hind quarters of another animal. All test animals died. Similar results were obtained by Bell, Clark and Cuthbertson (43), and by Rapport, Guild and Canzanelli (44) using similar techniques. These results were interpreted by the authors as demonstrating the release of a toxic factor by the traumatized extremities. However, this method of crosstransfusion does not prevent loss of blood volume from the test animal into the traumatized extremities, and therefore proves only that nervous factors are not necessary. Best and Solandt (45) and Chess, Chess and Cole (46) carried out crosstransfusion experiments using care to prevent the test dog bleeding into the traumatized dog. Since many of their test dogs died, they believed that a toxic factor was present.

In our experiments, care was taken to see that the test dog neither gained nor lost blood volume to the traumatized dog. Therefore, outside of the deleterious effect of the crosstransfusion procedure itself and the deliberate reduction of blood volume by bleeding some of the test dogs, the only factor that could operate to cause death in the test dogs was the addition of some toxic factor to the blood stream by the body or by the traumatized legs of the trauma dog or the excessive removal of some essential ingredient from the circulating blood by the tissues of the traumatized dog.

In the experiments presented in table 1A, three test animals died following the crosstransfusion with traumatized dogs. However, similar results were obtained in the control experiments. Furthermore, all test dogs maintained normal arterial pressures, throughout the crosstransfusion and for some hours afterwards. It is believed, therefore that the deaths which did occur resulted from complications introduced by the crosstransfusion technique, due perhaps to bacterial contamination, to the inability to avoid completely mismatching of the blood, or to changes in the blood as a result of its contact with the stromuhr.

Despite the added susceptibility conferred by the crosstransfusion technique, and despite the deliberate removal of sizable amounts of blood from three of

the test animals, eight out of twelve survived indefinitely. This is especially striking in the group receiving blood, directly from the vena cava of the traumatized dog (table 1D). *It is, therefore, concluded that these experiments clearly demonstrate that no toxic substance appears in the blood stream of traumatized dogs or is released by traumatized tissue which is capable of inducing a significant decline of arterial pressure or of causing death in a normal dog during the period of cross-transfusion. Furthermore, no essential ingredient is exhausted from the blood by the traumatized tissues which cannot be replaced by the normal dog.* The results of these experiments, however, do not rule out the possibility that either of the above mechanisms might play a contributory rôle in the traumatized dog in the presence of the slowing of the circulation induced by the loss of plasma into the traumatized extremities. These experiments also do not rule out the possibility that functional changes might have been produced in the test dog which were not recognized by the simple expedient of recording the mean arterial pressure.

#### SUMMARY

In 12 experiments both hind legs of a dog were traumatized by compressing them with rubber tubing wrapped in a continuous spiral from the ankle to the groin. This procedure rendered the legs completely ischemic. This dog, designated as the traumatized (shock) dog, was then crosstransfused with a test dog beginning 30 to 45 minutes before release of the rubber tubes and continuing usually until the death of the traumatized dog. In the various experiments, the crosstransfusion was maintained for 1.2 to 4.75 hours after release of the compression and amounted to 1.6 to 35.4 L. The crosstransfusion was carried out by means of a stromuhr which assured us that the test dog neither lost to nor gained blood volume from the traumatized dog. In 6 of the experiments all the blood returning from the traumatized extremities by way of the vena cava passed through the test dog before returning to the traumatized dog. All traumatized dogs died within 1.2 to 5.1 hours after release of the compression.

Eight of the test dogs survived indefinitely. The remaining 4 maintained their arterial pressure throughout the crosstransfusion and for several hour thereafter. Their ultimate death can, it is believed, be explained on technical grounds.

These experiments demonstrate that no toxic factor appears in the circulation in this form of trauma which can initiate shock. They render unlikely but do not rule out the possibility that a hypothetical factor might be present which could play a contributory rôle in a traumatized animal in the presence of a circulation impaired by severe loss of plasma and/or blood into the traumatized tissues.

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# THE ACTIVE PRINCIPLE OF PLACENTAL TOXIN: THROMBOPLASTIN; ITS INACTIVATOR IN BLOOD: ANTITHROMBOPLASTIN<sup>1</sup>

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The toxicity of placental extracts upon injection into experimental animals has been investigated repeatedly during the past half century (1, 3, 6, 7, 8, 9, 10, 12, 13, 14, 15, 19, 20, 21, 22, 23, 24, 30, 37, 38, 39, 40, 41, 42). This was for the purpose of finding the causative agent of toxemia of late pregnancy.

The writer first reported work with extracts of this kind in 1946. As in the past, the purpose was to contribute to our knowledge of toxemia of pregnancy.

A quantitative mouse assay was perfected for measuring the placental toxin (25, 28) and for measuring its inactivator (27) in blood. Using this assay, it was possible to show that the toxin which can be extracted from the mucosa of the progestational uterus is similar to or identical to the placental toxin (25). It was also found that the inactivator of the toxin is present in blood in higher concentration during pregnancy (29) than in normal blood. Despite this, pregnant mice are more sensitive to the toxin than are the non-pregnant animals (29). Following sub-lethal doses, the animals become temporarily desensitized to the toxin (28). It is possible to produce focal liver lesions consistently with these extracts (25, 28).

The work to be reported at this time is a continuation of previous experiments and shows that the toxic agent of these placental extracts is thromboplastin. It will also be shown that the serum factor which inactivated placental toxin is antithromboplastin.

**EXPERIMENTAL. Procedure.** The tissues from which extracts were to be prepared were perfused with saline until free of blood (27). They were then frozen until the time for extraction. After thawing, they were ground together with sand in a mortar, mixed with saline, and the supernatant fluid was clarified by centrifugation. The grinding and extraction were repeated with second and third portions of saline, and the three supernatants were combined. Finally one gram of tissue was represented by 4 cc. of extract. Extracts thus prepared and stored frozen maintained full activity for several months.

The toxin activity was measured with the mouse assay (25). This requires intravenous injection. Each of the tissue extracts is without effect when administered by other routes (25). The end point is prompt death. The arbitrary mouse unit of toxin is the minimum lethal dose (MLD) for mice of 20 grams' body weight (25, 28).

The thromboplastin activity was estimated by employing the one-stage prothrombin-time method. One-tenth cubic centimeter samples of oxalated human plasma were added to calcium chloride solution (0.1 cc., 0.28 per cent) and serial

<sup>1</sup> Aided by a grant from the National Institute of Health.

dilutions of the extracts (0.1 cc.). The calcium and extracts were mixed and the plasma was added to this mixture. A water bath of approximately 37°C (35-37°C) was used. The plasma was obtained from blood which had been mixed with 1.32 per cent sodium oxalate in the proportions 9:1. Only freshly drawn plasma was satisfactory because of the decreased clotting rates on storage. Pooled samples were collected and kept in the cold, or frozen, during the few hours over which high clotting rates could be obtained. The arbitrary unit of thromboplastin used in this work was the amount that caused clotting in 40 seconds.

TABLE 1  
*High-speed centrifugation of extracts*

BEFORE AND AFTER CENTRIFUGING	ACTIVITY, u/cc.					
	Thromboplastin			Toxin		
	Before	After		Before	After	
		Super-natant	Sediment		Super-natant	Sediment
Uterus, pseudopregnant, rabbit, 5 days.....	126	2	25	80	5	32
Placenta, human, term						
#1.....	112	3	28	90	3	36
#2.....	13	3	10	60	5	16
#3.....	38	2	25	55	4	12
#4.....	43	3	35	55	5	20
#5.....	102		13	90		56
Lung, rabbit						
#1.....	68	(0.2)*	19	200	10	50
#2.....	72	(0.1)*	46	80	7	48
Brain, rabbit						
#1.....	12	(0.1)*	1	16	<2	6
#2.....	13	(0.1)*	8	22	<2	16
Kidney, rabbit.....	100	(0.1)*	6	60	3	10
Liver, rabbit.....	10	(0.1)*	(0.2)*	10	2	<2
Skeletal muscle, rabbit.....	2.4	(0.02)*	0.6	3	<2	<2

\* Values by extrapolation.

RESULTS. Extracts of several tissues from rabbits including an extract of a "pseudo-pregnant" uterus (5 day positive Friedmann-test rabbit) are compared with human placental extract in figure 1. The placental tissue was among the rich sources of thromboplastin. This therefore confirms (24, 7) that the placental extracts which have previously been shown to be rich in toxin are also rich in thromboplastin. It might be mentioned that the curves for the extracts of liver and of kidney were peculiar in that an inhibitor appeared to be diluted out. Despite the influence of such extraneous factors, table 1 establishes that toxin and thromboplastin content parallel each other in extracts of several tissues including not only those rich in toxin (placenta and endometrium), but also the usual rich laboratory sources of thromboplastin, namely, brain and lung.

In the experiments of figure 2, samples of placental extract were inactivated by heating to different temperatures for one hour. Loss of thromboplastin activity closely paralleled the loss of toxic activity, the critical temperature being between 50–55°C. The pH of the extract was approximately 6.5, which is the usual acidity of the simple saline extracts. When made faintly alkaline, the critical temperature tended to be slightly higher.

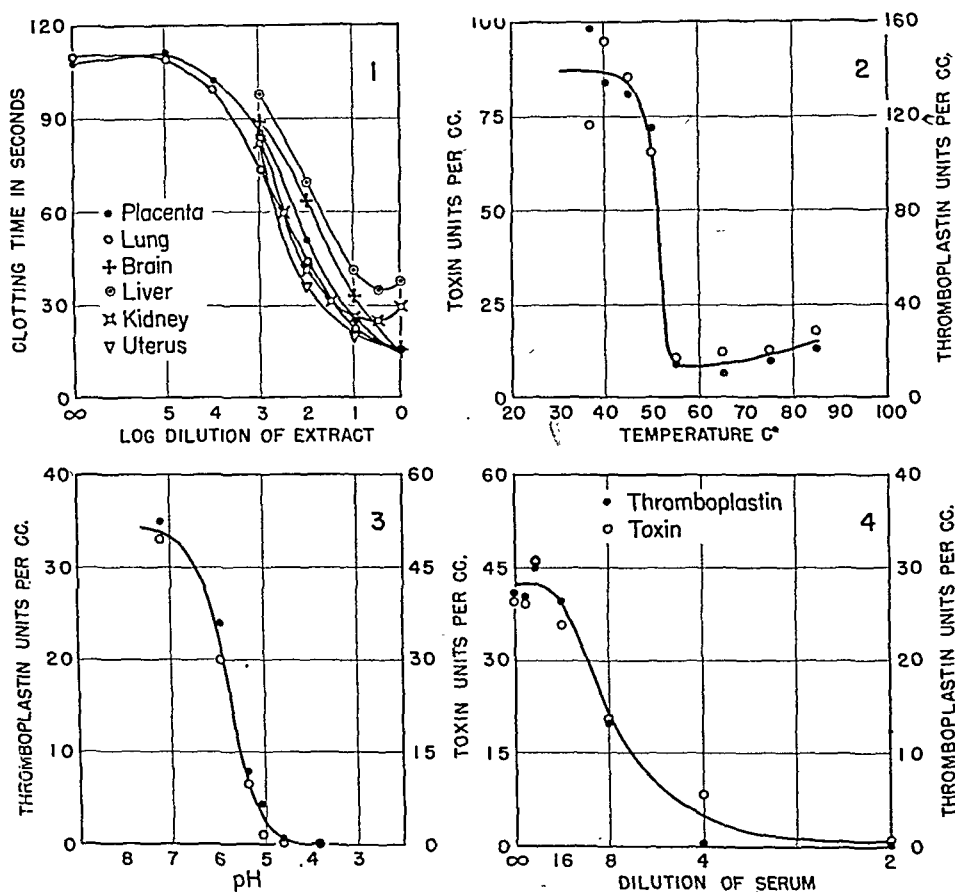


Fig. 1. Clotting times for recalcified plasma when mixed with different dilutions of tissue extracts.

Fig. 2. Inactivation of toxin and of thromboplastin by heat.

Fig. 3. Precipitation of toxin and of thromboplastin by acidification.

Fig. 4. Inactivation of toxin and of thromboplastin by serum.

Precipitation of a placental extract was also carried out by progressive acidification with 0.1 N. HCl. The supernatants were clarified by centrifugation, adjusted to neutrality, and assayed. The results (fig. 3) show that the thromboplastin and toxin activities disappeared in parallel upon acidification.

It is known that thromboplastin is of macro-molecular size (5); therefore, it was important to determine whether the toxin is also a macromolecule. After a preliminary centrifugation at 4,000 RPM, for fifteen minutes, to remove sediment which gradually separated out on storage, samples of extracts were centri-

fuged at 22-23,000 RPM in a multi-speed attachment (International Equipment Co.) for three hours. Then the original toxic extracts, the supernatant fluids, and the sediments were assayed in the clotting (thromboplastin) assay and in the mouse (toxin) assay (table 1). Both thromboplastin and toxin were sedimented out of solution regardless of the tissue source, the activities for placental and uterine extracts responding in the same manner as the brain and lung extracts. That sedimentation of the toxin was similar to that for thromboplastin, identifies it as a macromolecule or as associated with a macromolecule and is further consistent with the identity of the toxin with thromboplastin or of its adsorption in common with thromboplastin.

TABLE 2  
*Responses of mice to mixtures of tissue extracts and heparin*

	HEPARIN, U/CC. OF EXTRACT		
	0	1	10
Lethal dose of toxic extract, units:			
1. Placental extract, 40 u/cc.....	1	2.5	>20
2. Lung extract, 40 u/cc.....	1	2.1	>20

TABLE 3  
*Responses of heparin-treated mice to tissue extracts*

	HEPARIN, INTRAVENOUSLY, U/MOUSE		
	0	0.2	2
Lethal dose of toxic extract, units:			
1. Placental extract.....	1	2.2	15
2. Lung extract.....	1	2.5	>20

Since heparin blocks the blood clotting mechanism by blocking the activation of prothrombin, i.e., by blocking the formation of thrombin from prothrombin under the influence of thromboplastin (4), heparin should provide protection against placental toxin. This was found to be the case, as shown by two experimental approaches. Protection was obtained by mixing heparin with the extract before injection (table 2) as well as by preliminary intravenous injection of heparin (table 3). The degree of protection was a function of the dosage of heparin and the protection was effective whether the extract was prepared from placenta or from lung. With large doses of heparin, the animals were so completely protected that they showed no reaction at all even to massive doses (20 units) of extract. Thus a specific inhibitor of the blood clotting mechanism inhibited the toxic action of the extracts.

To determine if the capillary flow was altered by the injection of the extracts, the minute vessels of the ears of rabbits and of mice were observed, under a microscope at a magnification of 100 times, during and after injection of extracts of placenta, of pseudo-pregnant uterus, and of lung. If several lethal doses

of extract were injected into mice within 9–15 seconds, a momentary shower of emboli shot through the arterioles, then flow of blood stopped. There was temporary apnea, a gasping type of respiration and after 30–60 seconds respiration ceased. Following these large doses, the blood was clotted in the inferior vena cava, and in the portal vein. At 10–20 seconds after injection of *just-lethal* doses, the circulation in the vascular bed slowed down markedly and gradually stopped completely. Following this, within 40–60 seconds after the injection, respiratory movements ceased. Upon palpation and upon opening the chest cavity, the heart was still beating, usually regularly and forcefully. Blood in the large vessels was not clotted; it had a prolonged clotting time. With *just sub-lethal* doses, the circulation of the capillary bed slowed down markedly and most of it stopped, but then gradually resumed. Throughout, similar findings followed whether the injection were of placental, of pseudo-pregnant uterus, or of lung extract. Thus, while grossly the animals treated with the extracts appeared to have died a respiratory death, microscopic observation showed that they died of obstruction of the capillary vascular bed.

Several independent lines of experimental evidence, above, are not only consistent with but strongly support the proposition that placental toxin and thromboplastin are one and the same substance.

It is to be recalled that there exists in serum a powerful neutralizer (21, 22, 12) or inactivator (27) which detoxifies the extract whether from placenta (21, 7, 25), lung (7), endometrium (25), or skeletal muscle (25). If the toxin were thromboplastin, then this inactivator would be, by definition, antithromboplastin. Although there are reports of an antithromboplastin (35, 36), the experimental evidence is not especially convincing. Failure of an extract to kill a mouse, after toxin inactivation (by antithromboplastin contained in serum) is a far more dramatic experiment.

The serum was pooled from ordinary clotted blood of young men. In the experiment of figure 4, placental extract, serum, and saline were mixed in quantities sufficient to give the dilutions indicated, as in the method previously used to measure the toxin inactivator (27). The mixtures, adjusted to faint alkalinity to phenolsulphonphthalein, were incubated at 37°C for two hours or more. The results in figure 4 show how the serum destroyed the toxin and how it destroyed thromboplastin activity of placental extract. Both activities were destroyed by the serum regardless of whether the extracts were of placenta or of lung.

DISCUSSION. The experiments just described all confirm that thromboplastin is the toxic agent in the tissue extracts studied for many years (1, 3, 6, 7, 8, 9, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25–29, 30, 32–34, 37, 38, 39, 40, 41, 42). In this new work there is no evidence to the contrary.

It follows therefore that thromboplastin is actually the causative agent in toxemia of pregnancy or these extracts which have been so repeatedly experimented with have no bearing on the toxemia of late pregnancy.

The possibilities that thromboplastin might be in part at least responsible for toxemia of pregnancy is supported by the following facts.

1. The anatomic lesions of eclampsia are consistent with the underlying lesion

being multiple thromboses. Scattered hemorrhages in the central nervous system are not infrequently the immediate cause of death as can be confirmed in the literature (7) or by reviewing the pathological reports. The typical liver lesion of eclampsia is commonly associated with thromboses (30, 10, 2, 16, 7) and the acute lesion of the kidney is not uncommonly associated with thromboses (30, 31, 10, 2, 16, 7). Similarly, hepato-renal focal lesions (21, 3, 6, 26, 28, 29, 7) associated with thromboses, and scattered hemorrhages are found in experimental animals after intravenous administration of tissue extracts. (This does not take into account the question of periportal or mid-zonal necrosis as against focal necrosis.)

2. The concentration of antithromboplastin of the blood increases during pregnancy (29). To be without significance, this would have to be purely coincidental.

3. Anatomical relationship (11) does not preclude that thromboplastin from the placenta can enter the maternal blood stream.

4. Pregnant animals are more sensitive to thromboplastin than are non-pregnant animals (29).

5. Although recorded cases are few in number, in moribund toxemia patients the blood clotting time is prolonged or the blood fails to clot and fibrinogen is often decreased in concentration or is even absent (37).

6. There are two reports in the literature (21, 6) that the "neutralizer" in serum (therefore antithromboplastin) is markedly decreased in eclamptic patients.

These are all strong arguments in favor of thromboplastin as the chemical mediator of toxemia of pregnancy, therefore the cautious administration of heparin or of antithromboplastin, if it could be prepared for use, would be indicated in the pre-eclamptic state, but for the present it will be sufficient to have called attention to these possibilities.

There is no sound argument against the possibility of thromboplastin being the causative agent of toxemia of pregnancy. Three suggestions might be discussed however.

1. An H-substance (3) has been suggested as the causative agent of eclampsia, but its true relationship to toxemia of pregnancy, if any, is yet to be established.

2. Placental protein autolysates (2, 3) as a basis of toxemia of pregnancy are subject to the same criticism.

3. "Necrosin" (32-34) has been suggested as the causative agent of toxemia of pregnancy but an etiologic relationship to toxemia of pregnancy has not been established and therefore it is not precluded that thromboplastin is the causative agent.

#### SUMMARY

1. Tissue extracts, especially placental extracts, have long been known to possess a toxin and the toxin has many times been regarded as a causative agent in toxemia of pregnancy.

2. It has been shown that this toxin is thromboplastin and that its lethal effect depends on intravascular clotting.

3. The inactivator of this toxin is antithromboplastin.

4. The possibilities that thromboplastin might be the cause of toxemia of pregnancy are reviewed. Actual proof is lacking, but there is no evidence to the contrary.

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# THE RENAL CLEARANCE OF ESSENTIAL AMINO ACIDS: ARGININE, HISTIDINE, LYSINE AND METHIONINE

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The extent to which the kidney tubules are able to reabsorb specific amino acids at elevated plasma levels is a factor to be considered in the clinical use of protein hydrolysates or mixtures of amino acids.

Reasons have been presented whereby the desirability of re-evaluating and extending renal clearance data of the amino acids by employing the recently devised microbiological methods of determination were indicated (Beyer *et al.*, 1946). These methods have the advantage that they are based on the physiologic specificity of individual amino acids rather than on chemical reactivity that may be common to more than one amino acid or type of compound.

This paper is concerned with the renal clearances of arginine, histidine, lysine, and methionine. Renal clearance data for methionine and lysine have not been reported previously. Studies in which chemical assay methods were employed have been made of the renal clearances of arginine (Pitts, 1944) and histidine (Doty, 1941).

**METHODS.** The general procedure for the experiments and the conditions for individual studies are given in the protocols listed as tables 1 to 4. The dogs employed were in a post-absorptive state. Prior to each test about 500 cc. of water were administered by stomach tube to the dog to insure an adequate urine flow throughout the course of the experiment. Similarly, 3 grams of creatinine were injected subcutaneously prior to the test and an additional one gram of creatinine was administered at about the midpoint of the experiment in order to maintain plasma levels of the compound adequate for renal clearance determinations. In addition 150-200 mgm. of para-aminohippuric acid were administered *per os* to the dogs prior to most of the tests for the measurement of renal plasma flow. Subsequent experiments failed to show any consistent effect of amino acid administration on the renal plasma flow.

Our aim has been to determine the renal clearance of the individual amino acids at normal post-absorptive blood levels followed by similar clearance periods subsequent to elevation of the blood level in increments by the use of an initial priming dose and constant intravenous infusion of the amino acid under investigation. Clearance periods were of 10 minutes' duration. Following the elevation of the blood level of an amino acid to a new value a period of 15 to 20 minutes was allowed for equilibration of the plasma concentration before additional clearances were obtained. An attempt has been made in each instance to exceed the maximal rate of tubular reabsorption of the amino acids studied.

Additional details of the procedures employed in our renal clearance studies

have been described in previous publications from these laboratories (Beyer *et al.*, 1946).

At least two experiments using each amino acid were performed. Excellent agreement between the results of separate experiments was obtained. Three dogs were used in the course of the work.

Arginine, histidine, and methionine were determined microbiologically by the procedure of Stokes *et al.*, (1945) employing *Streptococcus fecalis R* as the assay organism. The extent of bacterial growth was determined turbidimetrically after an incubation period of 18-24 hours. Lysine was determined essentially

TABLE 1

*Renal clearance studies for l(+)-arginine: protocol*

Dog 1, wt. 16.1 kgm.

TIME	RENAL PLASMA FLOW PAH	CREATININE CLEARANCE	URINE FLOW	l(+)-ARGININE				
				Plasma conc.	Amount filtered	Amount reabsorbed	Amount excreted	Clearance
Control: Post-absorptive but after priming dose of water								
hr:min.	cc./min.	cc./min.	cc./min.	mgm./cc.	mgm./min.	mgm./min.	mgm./min.	cc./min.
0:10	231.7	79.5	2.90	0.031	2.46	2.46	0.004	0.13
0:20	216.0	70.2	3.85	0.018	1.26	1.25	0.005	0.33
Priming 2.0 mgm./kgm.—Maintenance 2.0 mgm./kgm./min.—Infusion 3 cc./min.								
0:50	205.8	77.8	4.90	0.112	8.71	8.70	0.010	0.09
1:00		78.3	4.90	0.153	11.98	11.96	0.017	0.11
Priming 3.0 mgm./kgm.—Maintenance 6.0 mgm./kgm./min.—Infusion 3 cc./min.								
1:30		68.1	5.60	0.338	23.02	12.03*	10.99	28.3
1:40	177.5	57.8	5.80	0.458	26.47	12.80*	13.67	29.8
Priming 4.0 mgm./kgm.—Maintenance 10 mgm./kgm./min.—Infusion 3 cc./min.								
2:10	174.6	60.7	6.64	0.680	41.28	10.75*	30.53	44.9
2:20		63.6	6.78	0.720	45.79	8.42*	37.37	51.9

\* T<sub>m</sub> = 12.02, 12.80, 10.75 and 8.42 (average 11.00) mgm./min.

according to the method of Dunn *et al.* (1944) with *Leuconostoc mesenteroides* P-60 as the assay organism. The response to lysine was determined turbidimetrically after 18-24 hours of incubation.

Microbiologic assays of the urine samples were carried out directly without previous treatment. Determinations on plasma were carried out on protein-free filtrates prepared according to the method of Dunn *et al.*, (1945). Added arginine, histidine, lysine, and methionine could be recovered from blood plasma to the extent of 97 per cent, 94 per cent, 107 per cent, and 93 per cent respectively.

The natural forms of arginine, histidine, and lysine were used both in the infusion experiments and in the microbiologic standards. Accordingly, the

values presented for these amino acids were in terms of the natural product. Racemic methionine was used in the infusion experiments, as a standard in the microbiologic determinations, and in the calculation of the results. It is appreciated that *Streptococcus fecalis R* is capable of utilizing only l-(+)-methionine and that the unnatural enantiomorph may have influenced the fate of l-(+)-methionine within the kidney.

RESULTS AND DISCUSSION. At post-absorptive blood levels of arginine and lysine less than 0.5 per cent of either amino acid calculated as filtered at the glomerulus appeared in the urine (Tables 1 and 2). As the blood levels of either

TABLE 2  
*Renal clearance studies for l(+)-lysine: protocol*

Dog 1, wt. 16.3 kgm.

TIME	CREATININE CLEARANCE	URINE FLOW	l(+)-LYSINE				
			Plasma conc.	Amount filtered	Amount reabsorbed	Amount excreted	Clearance
Control: Post-absorptive state but after priming dose of water							
<i>hr:min.</i>	<i>cc./min.</i>	<i>cc./min.</i>	<i>mgm./cc.</i>	<i>mgm./min.</i>	<i>mgm./min.</i>	<i>mgm./min.</i>	<i>cc./min.</i>
0:10	60.5	2.55	0.079	4.78	4.77	0.011	0.14
0:20	62.5	2.50	0.065	4.06	4.05	0.009	0.14
Priming 2.0 mgm./kgm.—Maintenance 2.5 mgm./kgm./min.—Infusion 3 cc./min.							
0:50	64.2	3.80	0.169	10.85	10.82*	0.027	0.16
1:00	59.9	4.10	0.222	13.30	13.08*	0.224	1.01
Priming 3.0 mgm./kgm.—Maintenance 5.0 mgm./kgm./min.—Infusion 3 cc./min.							
1:28	64.8	4.85	0.449	29.10	14.30*	14.80	33.0
1:38	60.4	4.75	0.508	30.68	13.59*	17.09	33.6
Priming 4.0 mgm./kgm.—Maintenance 7.0 mgm./kgm./min.—Infusion 3 cc./min.							
2:07	53.7	5.40	0.764	41.03	11.39*	29.64	38.8
2:17	57.1	6.38	0.869	49.62	15.03*	34.59	39.8

\* T<sub>m</sub> = 10.82, 13.08, 14.30, 13.59, 11.38 and 15.03 (average 13.20) mgm./min.

amino acid were raised, however, sharp breaks in the calculated clearances of the compounds were observed. The data indicated that at the higher blood levels the maximal rate of tubular reabsorption of each amino acid had been exceeded and a T<sub>m</sub> of 11.0 mgm./min. for arginine and 13.1 mgm./min. for lysine were obtained.

The renal clearances for both histidine and methionine at all blood levels studied were 0.8 cc./min. or less (Tables 3 and 4). Even at blood levels of histidine over 10 times that of the post-absorptive state, more than 99 per cent of the amino acid calculated as filtered was reabsorbed. Similarly, at methionine blood levels of over 90 times that of the post-absorptive state more than 99 per cent of the amino acid calculated as filtered was reabsorbed. There was no indication with either histidine or methionine that the maximal rate of

TABLE 3

*Renal clearance studies for l(+)-histidine: protocol*

Dog 1, wt. 16.0 kgm.

TIME	RENAL PLASMA FLOW PAH	CREATININE CLEARANCE	URINE FLOW	l(+)-HISTIDINE				
				Plasma conc.	Amount filtered	Amount reabsorbed	Amount excreted	Clearance
Control: Post-absorptive but after priming dose of water								
hr:min.	cc./min.	cc./min.	cc./min.	mgm./cc.	mgm./min.	mgm./min.	mgm./min.	cc./min.
0:10	194.4	83.6	0.70	0.031	2.59	2.58	0.005	0.15
0:20	165.5	67.5	0.50	0.030	2.02	2.02	0.005	0.15
Priming 2.0 mgm./kgm.—Maintenance 3.0 mgm./kgm./min.—Infusion 3 cc./min.								
0:50	182.0	79.2	1.20	0.138	10.93	10.91	0.019	0.14
1:00	178.3	78.7	2.40	0.159	12.51	12.48	0.027	0.17
Priming 3.0 mgm./kgm.—Maintenance 5.0 mgm./kgm./min.—Infusion 3 cc./min.								
1:30	167.0	72.1	4.55	0.324	23.36	23.27	0.091	0.28
1:40	193.8	82.9	5.70	0.416	34.49	34.32	0.171	0.41

TABLE 4

*Renal clearance studies for dl-methionine: protocol*

Dog 2, wt. 23.4 kgm.

TIME	RENAL PLASMA FLOW PAH	CREATININE CLEARANCE	URINE FLOW	dl-METHIONINE				
				Plasma conc.	Amount filtered	Amount reabsorbed	Amount excreted	Clearance
Control: Post-absorptive but after priming dose of water								
hr:min.	cc./min.	cc./min.	cc./min.	mgm./cc.	mgm./min.	mgm./min.	mgm./min.	cc./min.
0:05	336	103.9	2.35	0.009	0.94	0.93	0.006	0.70
0:15	389	100.6	3.65	0.014	1.41	1.40	0.010	0.74
Priming 2.0 mgm./kgm.—Maintenance 2.0 mgm./kgm./min.—Infusion 6 cc./min.								
0:45	278	89.1	6.20	0.147	13.10	12.98	0.12	0.79
0:55	283	94.3	6.85	0.208	19.61	19.46	0.15	0.72
Priming 3.0 mgm./kgm.—Maintenance 5.0 mgm./kgm./min.—Infusion 6 cc./min.								
1:25	267	83.2	5.70	0.365	30.37	30.23	0.14	0.39
1:35	263	84.1	5.10	0.495	41.63	41.45	0.18	0.36
Priming 3.0 mgm./kgm.—Maintenance 7.0 mgm./kgm./min.—Infusion 6 cc./min.								
2:06	223	84.5	1.6	0.960	81.12	80.73	0.39	0.41
2:16	254	98.3	1.2	1.150	113.04	112.44	0.60	0.52

tubular reabsorption had been reached. It was calculated that at least 30 mgm. of histidine per minute and 100 mgm. of methionine per minute could be reabsorbed by the kidneys of the dog. It was impractical to administer additional

amounts of histidine and methionine since even the quantities used caused some nausea.

The results of the present and preceding investigations (Beyer *et al.*, 1946) have emphasized the existence of marked differences between the amino acids with respect to their rates of tubular reabsorption. Histidine, methionine, leucine, isoleucine, tryptophane, and valine are representatives of amino acids readily reabsorbed by the tubule and whose  $T_m$  cannot be reached by the administration of amounts of these amino acids that do not cause severe nausea. On the other hand arginine and lysine are characterized by having a much lower renal threshold and a measurable  $T_m$ .

Although previous investigators have reported individual differences among the amino acids with respect to rate of tubular reabsorption (Eaton and Doty, 1941; Doty, 1941; Kriss, 1939; Pitts, 1944) certain of the data may be criticized because of the non-specific analytical methods employed. For example, it seems reasonable that at very high plasma concentrations of one amino acid there could be sufficient impedance of the absorption of related amino acids to increase incommensurately the UV values when results were based upon  $\alpha$ -amino nitrogen determinations. Clearance values in excess of that of the specific compound under examination consequently might result.

Conversely, the use of microbiologic methods of determination is open to the practical disadvantage that, while the methods are quite specific, plasma and urine must be examined for a large number of amino acids in order to obtain information concerning the influence of the reabsorption of one amino acid on the corresponding reabsorption of unrelated amino acids.

#### SUMMARY

Renal clearance studies of arginine, histidine, lysine, and methionine in dogs indicated individual differences in the tubular reabsorption of these amino acids. At plasma levels up to 10 and 50 times respectively that of the post-absorptive state reabsorption of histidine and methionine essentially was complete and no evidence was available that the maximal rate of tubular reabsorption had been reached. The capacity for tubular reabsorption of arginine and lysine was markedly less than that for histidine and methionine.  $T_m$  values were readily obtainable and were found to be in the order of 11 mgm./min. for arginine and 13 mgm./min. for lysine.

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# AUDIOGENIC FITS PRODUCED BY MAGNESIUM DEFICIENCY IN TAME DOMESTIC NORWAY RATS AND IN WILD NORWAY AND ALEXANDRINE RATS<sup>1</sup>

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McCollum and Orent (1), reported in 1931 that young, tame domestic rats fed a diet deficient in magnesium soon showed alarm and apprehension in the presence of high-pitched noises. This hyperirritability increased from day to day, until after 11 to 14 days convulsions occurred. The convulsive pattern consisted of violent, circular running, followed by tonic-clonic spasm and coma. About 86 per cent of the animals died during the first fit.

It is well known that essentially the same type of fit occurs in some apparently normal domestic rats following stimulation by air blast, key jingling, electric bells or buzzers (2). In certain colonies a large percentage of rats show these fits, while in other colonies only a small number or none show them. This variation in susceptibility to audiogenic seizures remains unexplained. In contrast to audiogenic fits induced in animals suffering from magnesium deficiency, fits occurring in normally fed colonies are seldom fatal (3).

Wild Norway and Alexandrine rats taken directly from the city streets did not show audiogenic fits (2). Their response to the auditory stimulus differed markedly from that of domestic Norway rats. They attacked and bit at the source of stimulation, while the domestic rats retreated in an obvious state of fear.

The present experiments were undertaken to determine whether the resistant wild rats fed the magnesium-deficient diet have audiogenic fits of the same type and intensity as those shown by the domestic rats.

**METHODS.** Of the 62 young rats studied, 34 were fed the magnesium-deficient diet (see below); of these 16 were tame domestic, 10 wild Norway and 8 wild Alexandrine rats. The remaining 12 domestic, 8 wild Norway and 8 wild Alexandrines served as controls.

The wild Norways were trapped in Baltimore; the wild Alexandrines were the offspring of rats trapped in Florida;<sup>3</sup> the domestic rats were albinos of the Wistar strain that do not have audiogenic fits when fed our stock diet.

The rats, both wild and domestic, were housed in individual cages with a 100 cc.

<sup>1</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and The John Hopkins University, and aided by support from the Epilepsy Medical Research Fund.

<sup>2</sup> Now at the University of Mississippi.

<sup>3</sup> The wild Norways were trapped by the Rodent Control Division of the City of Baltimore; the wild Alexandrines were provided by Mr. Jack Spencer of the Fish and Wildlife Service in Gainesville, Florida.

graduated, inverted water bottle and a nonspillable food cup. The cages had coarse-screened bottoms which prevented coprophagy. Food and water intake were recorded daily and body weights weekly. All rats were young, weighing between 28 and 41 grams at the start of the experimental period.

Three diets were used during the course of the experiment:

a. *Stock diet* (per 1000 grams)

Graham flour .....	725 grams
Skim milk .....	100 grams
Casein (Labco) .....	100 grams
Calcium carbonate .....	15 grams
Sodium chloride .....	10 grams
Butter .....	50 grams

b. *Magnesium-deficient diet* (per 1000 grams)<sup>4</sup>

Vitamin-free casein .....	180 grams
Dextrin .....	666 grams
Butterfat .....	80 grams
Salts #62 <sup>5</sup> .....	59 grams
Lederle B complex .....	15 grams

With the addition of:

Choline chloride .....	1 gram
Oleum percomorphum .....	13 drops

c. *Magnesium control diet* (per 1000 grams)

Magnesium sulphate .....	1 gram
(added to magnesium-deficient diet)	

The rats were kept on the stock diet for 10 days before being placed on either the magnesium-deficient or magnesium control diet. Audiogenic tests were given every day while the rats were on the stock diet and every other day while they were on the experimental and control diets. The tests were continued either until the rats died or until they gave sufficient indication of living for a long time (17 nonfatal tests for domestic, 25 to 27 for wild rats).

For testing, each rat was exposed for 2 minutes to a hissing air blast in a wire-mesh cage 12 by 12 by 20 inches, padded with felt on the sides and bottom to prevent injury. The metal nozzle of the air-blast hose was inserted through horizontal slits in the sides of the cage and moved around so as to remain at all times in close proximity to the rat's ear. Wild rats were taken from their living cages in metal traps, and transferred without handling into the testing cage through a specially constructed sliding door. The tame rats were transferred by hand.

**RESULTS.** *Domestic Norway rats.* Figure 1A shows typical records of a domestic rat on the magnesium-deficient diet. During the 10-day period on the stock diet none of the daily tests brought out any fits. On the magnesium-deficient diet, when the rat was tested every other day, the first fit occurred on the 10th day, and the second on the 12th day. This fit terminated in death.

<sup>4</sup> Recommended by Dr. Virginia Evans, Biochemical Department, School of Hygiene and Public Health, The Johns Hopkins University; a modification of a diet used by Sullivan and Evans. (4).

<sup>5</sup> Baker's Analyzed Salts, obtained from Arthur H. Thomas Company.

Figure 1B shows a typical record of one of the control rats. This animal did not have any fits, either during the 10-day period on the stock diet or during a 34-day period on the magnesium control diet.

Table 1A summarizes the results. All 16 rats on the deficient diet died during a fit. The first fit appeared after an average of 8 days on the diet. Some of the rats had fits as early as the 3rd and 4th days; a few not until the 15th or 16th days. Some of them died during their first fit; none survived more than 4 fits.

None of the 12 rats on the control diet had any fits during the 34 days that they were tested.

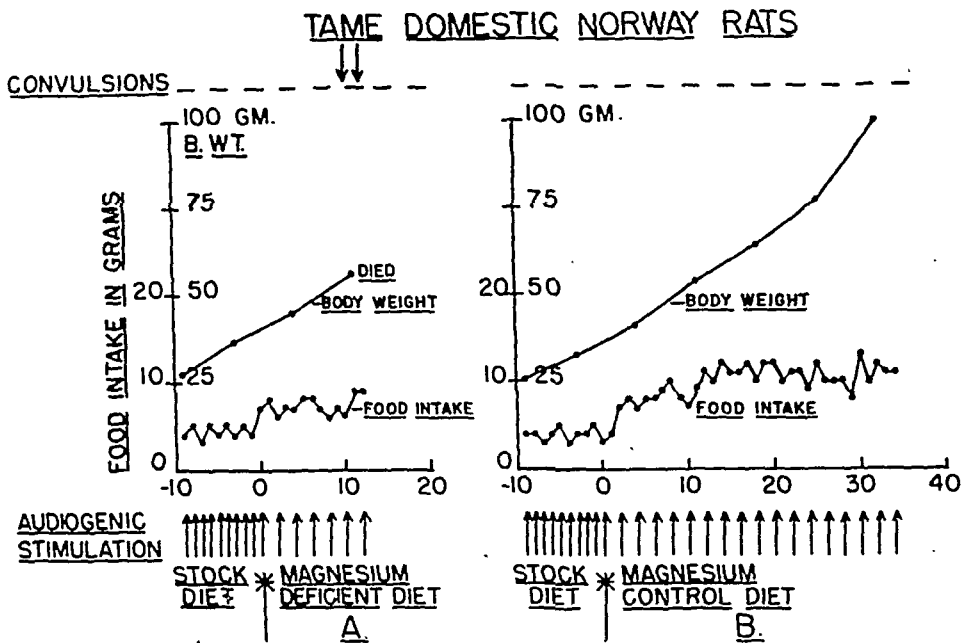


Fig. 1, A and B. Graph showing typical records of two tame domestic Norway rats, one on a magnesium-deficient diet, the other on the magnesium control diet. The ordinates show food intake and body weight in grams and the abscissas time in days. The arrows at the bottom show days on which the rats were exposed to the sound blast; the arrows at the top, the appearance of convulsions. The magnesium-deficient rat had its first fit on the 10th day and died during the second fit 2 days later. The control rat had no fits during the 34-day observation period.

*Wild Norway rats.* Figure 2A shows a typical record of one of the wild Norway rats kept on the magnesium-deficient diet. During the 10-day control period on the stock diet the audiogenic stimulation did not elicit any fits. On the deficient diet the rat had its first fit on the 6th day and thereafter had 5 more fits during a 55-day period. The experiment terminated after 55 days on the diet when it seemed unlikely that the rat would die during a subsequent fit. Figure 2B shows a typical record of one of the control wild Norway rats. This animal showed no fits, either during the 10-day period on the stock diet or during a 52-day period on the magnesium control diet.

Table 1B summarizes the results. The 10 wild Norways had their first fits 15 days, on the average, after the start of the deficient diet, with a wide range of 5 to 30 days. The rats were tested 26 or 27 times over a 52- to 55-day period. During this time they averaged 10 fits. None of the fits was fatal.

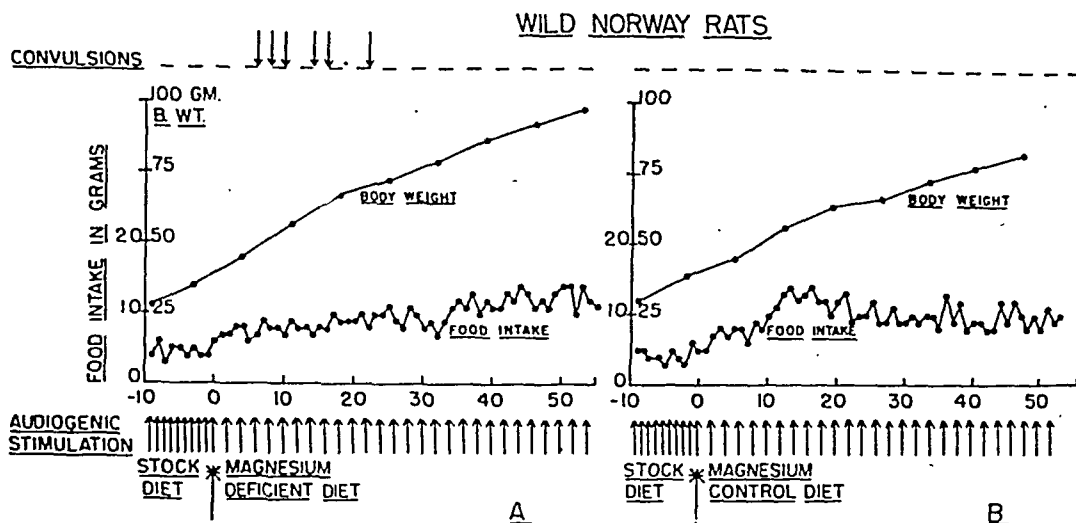


Fig. 2, A and B. Typical records for wild Norway rats, one on the magnesium-deficient diet, the other on the control diet. Legends the same as in figure 1. The magnesium deficient rat had 6 fits during the first 20 days, thereafter none. The control rat had no fits during the 52-day observation period.

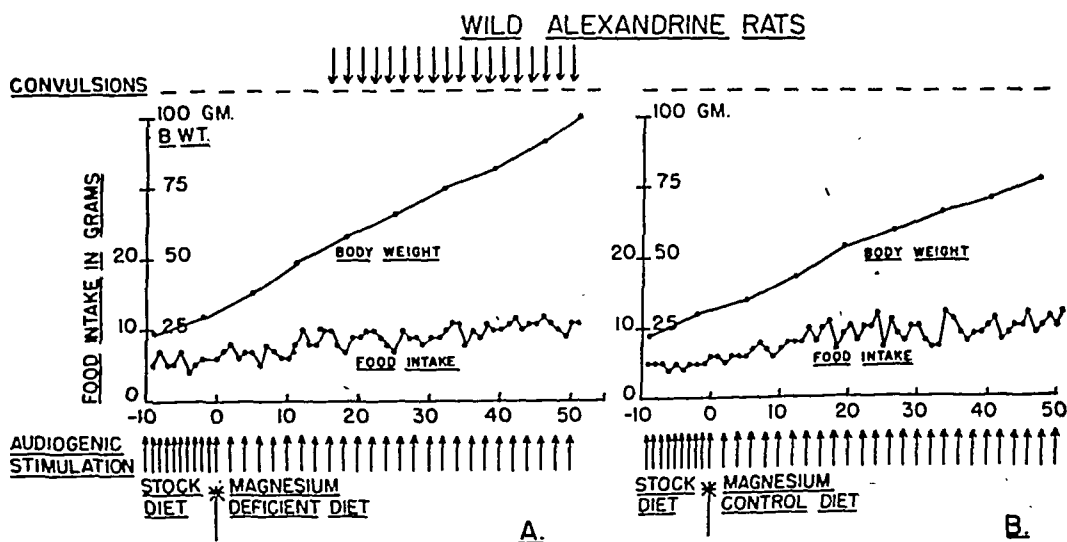


Fig. 3, A and B. Typical records for 2 Alexandrine rats. The magnesium-deficient rat did not have any fits until the 16th day; thereafter it had a fit with each stimulation. The control rat did not have any fits during the 50-day observation period.

Eight control wild Norways, tested on alternate days for a comparable time, had no fits at all.

*Wild Alexandrine rats.* Figure 3A gives a typical record of an Alexandrine rat kept for a 10-day period on the stock diet and for a 52-day period on the mag-

TABLE 1  
Audiogenic fits  
(Rats tested on alternate days)

RATS ON MAGNESIUM-DEFICIENT DIET						RATS ON MAGNESIUM CONTROL DIET				
Rat	Wt. at start of diet	Times tested	Appearance of first fit	No. of fits	Total period on diet	Rat	Wt. at start of diet	Times tested	No. of fits	Total period on diet

A. Tame Norways .

	gms.		days		days		gms.			days
1 ♂	41	6	9	2	12 (died)	17 ♂	38	17	0	34
2 ♂	38	5	9	1	10 (died)	18 ♂	38	17	0	34
3 ♀	38	14	15	3	28 (died)	19 ♀	38	17	0	34
4 ♀	37	5	3	4	10 (died)	20 ♀	40	17	0	34
5 ♀	38	10	9	3	10 (died)	21 ♀	40	17	0	34
6 ♂	38	5	5	3	10 (died)	22 ♂	40	17	0	34
7 ♀	40	8	5	4	16 (died)	23 ♀	38	17	0	34
8 ♂	38	7	8	3	14 (died)	24 ♀	40	17	0	34
9 ♀	40	8	6	4	16 (died)	25 ♀	40	17	0	34
10 ♀	38	14	16	3	28 (died)	26 ♂	37	17	0	34
11 ♀	38	5	4	4	10 (died)	27 ♂	40	17	0	34
12 ♀	38	10	10	5	20 (died)	28 ♂	38	17	0	34
13 ♂	38	5	6	3	10 (died)					
14 ♂	37	5	10	1	10 (died)					
15 ♀	38	7	10	3	14 (died)					
16 ♂	41	6	10	2	12 (died)					
Average ..	39	8	8	3	14		39	17	0	34

B. Wild Norways

1 ♂	41	26	18	12	52 (survived)	11 ♀	43	26	0	52
2 ♂	41	26	9	11	52 (survived)	12 ♂	38	26	0	52
3 ♀	40	26	5	6	52 (survived)	13 ♂	38	26	0	52
4 ♀	40	26	29	9	52 (survived)	14 ♀	41	26	0	52
5 ♂	36	26	11	7	52 (survived)	15 ♀	41	26	0	43
6 ♀	40	27	30	9	55 (survived)	16 ♀	42	26	0	43
7 ♀	40	27	6	6	55 (survived)	17 ♂	38	26	0	43
8 ♂	41	27	19	12	55 (survived)	18 ♂	39	26	0	43
9 ♂	36	27	12	7	55 (survived)					
10 ♂	28	27	12	18	55 (survived)					
Average ..	38	27	15	10	54		40	26	0	48

C. Wild Alexandrines

1 ♂	36	26	13	16	52 (survived)	9 ♂	38	26	0	52
2 ♂	35	26	13	19	52 (survived)	10 ♀	32	26	0	52
3 ♀	36	26	23	14	52 (survived)	11 ♂	38	26	0	52
4 ♀	38	26	9	17	52 (survived)	12 ♀	33	26	0	52
5 ♀	36	25	24	14	50 (survived)	13 ♀	37	26	0	52
6 ♀	38	25	11	17	50 (survived)	14 ♀	33	26	0	50
7 ♂	35	25	14	19	50 (survived)	15 ♂	38	26	0	50
8 ♂	36	25	14	16	50 (survived)	16 ♂	37	26	0	50
Average ..	36	26	15	17	51		36	26	0	51

nesium-deficient diet. It had no fits while on the stock diet but had frequent fits on the deficient diet, the first occurring on the 16th day. The experiment was terminated on the 52nd day. A sample record of one of the control rats is shown in figure 3B. This animal showed no fits during a 10-day period on the stock diet and none during a 50-day period on the magnesium control diet.

Table 1C summarizes the results. The 8 deficient rats had their first fits after an average of 15 days and had an average of 17 fits, none of which ended with the death of the animal.

None of the 8 rats on the magnesium control diet had fits at any time.

**DISCUSSION.** The results of these experiments give further proof of the importance of the rôle played by magnesium in the nutrition of the nervous system, particularly those parts concerned with the production of audiogenic fits. Every rat on the magnesium-deficient diet, the very resistant wild Norway and Alexandrine as well as the tame domestic Norways, had fits; on the other hand, none of the rats on the control diet had any fits at all.

The fits appeared sooner in the tame Norways than in either of the two wild forms, after an average interval of 8 days in the tame rats, and 15 days in the wild Norway and Alexandrine rats. The previous food of the tame Norways may have contained less magnesium than did that of the wild Norways (bones, seed, etc., which they may have eaten in the city yards and cellars), or the tame rats may have lost some of their ability to store magnesium as effectively as their wild ancestors.

The domestic rats did not survive more than 4 fits. In some instances they died during the first fit. Most of them died during the third fit. In marked contrast, the wild rats survived many fits, and not one died during a fit. Since the fits of the wild and domestic rats did not seem to differ in intensity, a reduced ability to withstand the violence of the fits may account for the death of the tame rats. The tame rats have much smaller adrenals than do the wild rats, and so may have less ability to withstand the severe strain imposed by the fits.

The wild Alexandrine rats had more fits than did the wild Norways. After the first fit, some of the rats had fits every time that they were tested. Compared to wild Norways, the wild Alexandrines are less fierce and pugnacious. Since, in general, domestic rats seem to be very susceptible to fits, the greater gentleness of the wild Alexandrine as compared to the wild Norway may be related in some way to the higher frequency of their fits.

#### SUMMARY

1. Domestic and wild Norway rats and wild Alexandrine rats on a magnesium-deficient and control diet were tested on alternate days with an air blast for susceptibility to audiogenic seizures. All the rats on the magnesium-deficient diet had fits, while not one of the control animals had a fit.

2. Domestic Norway rats fed the magnesium-deficient diet began to have running fits in an average of 8 days (range 3 to 16); wild Norways in an average of 15 days (range 5 to 30); and wild Alexandrines also in an average of 15 days (range 9 to 24).

3. Magnesium-deficient domestic Norways died usually during their first fit, and none survived more than 4 fits. Wild Norways and Alexandrines had between 6 and 19 fits, but none of them died during the entire test period of 50 to 55 days.

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# FURTHER OBSERVATIONS ON FACTORS INFLUENCING THE BIOCHEMICAL APPRAISAL OF NUTRITIONAL STATUS

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In a previous paper (1), it was pointed out that since the immediately preceding dietary intake of a nutrient was probably the most important factor in determining the level at which that nutrient would be excreted in the fasting urine, then it is possible for a high excretion level to be caused by a recent but transient high intake, thus perhaps giving false assurance of the general state of nutrition. Conversely, a recent but transient low intake of the nutrient may cause a low excretion level, thus promoting the inference that nutritional deficiency is imminent. The data in that report were obtained when young men received an adequate diet which contained relatively constant levels of nutrients from day to day, varying only to the extent customarily encountered where there is a wide choice of foods daily.

In the present report, there is discussed lack of correlation that may be found to occur between urinary excretion levels and frequently used measures of nutritional state such as physical and psychomotor performance. In the present instance, the intake of nutrients, rather than being relatively constant, was changed abruptly on two occasions (a) from normal levels to low levels and (b) from the low levels back to normal or above by means of crystalline nutrients received orally or intravenously. It will be shown that because of the lack of correlation between urinary excretion levels and performance, it is highly advisable to use all three types of data (clinical, biochemical and dietary) for the appraisal of nutritional state, except when gross specific signs would permit certain diagnosis of the deficiency state.

**PROCEDURE AND METHODS.** The data described in this report were obtained during the course of an investigation of the effects of a moderately restricted intake of B-complex vitamins and protein.<sup>1</sup> The investigation was carried out over a period of 50 weeks with seven volunteer subjects who, after an initial twelve weeks on a normal dietary regime, then consumed for the ensuing 35 weeks a diet containing levels of B-complex vitamins ranging from  $\frac{1}{3}$  to  $\frac{1}{6}$  those found in the normal diet. The level of protein intake was approximately  $\frac{1}{2}$  that found in the normal diet, but almost completely non-animal in type. The nutritive content of the normal and experimental diets is shown in table 1. Two of the subjects were chosen as "controls," and received supplements<sup>2</sup>

<sup>1</sup> Previously reported, this journal (3, 4).

<sup>2</sup> The supplements were as follows:

- (1) 40 grams animal protein (as 45 grams calcium caseinate)
- (2) 0.70 gram calcium as dicalcium phosphate
- (3) 0.54 gram phosphorus as dicalcium phosphate
- (4) 20 mgm. iron as iron pyrophosphate

in the form of tablets and capsules, while the remaining 5 subjects received placebos that were identical in appearance. The intake of nutrients by the control subjects equalled or slightly exceeded that of the normal diet period, while the intake of nutrients by the experimental subjects remained considerably less, as mentioned above, and as shown in table 1.

From time to time during the 35-week period when the experimental diet was consumed, the 5 experimental subjects received additively the supplements received by the controls.<sup>1</sup> The first supplement, thiamine, was given following 15 weeks of the restricted intake of nutrients, and thereafter additions were made at varying periods of time in the following sequence (2) protein—40 grams animal in type (calcium caseinate), (3) nicotinamide, (4) riboflavin,

TABLE 1  
*Nutritional content of the normal and experimental diets*

NUTRIENTS		NORMAL DIET	EXPERIMENTAL DIET
Calories.....		3,170*	3,300*
Protein.....	grams	70*	40
(1-Tryptophane).....	mgm.	700-900	210-300
Calcium.....	grams	0.86*	0.20*
Phosphorus.....	grams	1.26*	0.58*
Iron.....	mgm.	15.5*	12.0*
Thiamine.....	mgm.	1.44	0.50
Riboflavin.....	mgm.	1.84	0.30
Niacin.....	mgm.	15.6	5.8
Biotin.....	mcg.	44	19
L-casei factor.....	mcg.	64	23
Pantothenic acid.....	mgm.	4.7	1.1
Pyridoxine.....	mgm.	1.7	1.1
Ascorbic acid.....	mgm.	105*	90*
Vitamin A.....	I.U.	7,400*	16,600*

\* Calculated.

and (5) "lesser-known" B-complex factors, folic acid, pyridoxine, pantothenic acid, biotin, choline. The amount of supplementation was that shown for the control subjects,<sup>1</sup> plus in the case of thiamine, nicotinamide and riboflavin, larger amounts given on several occasions (3).

- (5) 666 I.U. vitamin D
- (6) 1.2 mgm. thiamine hydrochloride
- (7) 1.5 mgm. riboflavin
- (8) 12 mgm. nicotinamide
- (9) 60 mcg. biotin
- (10) 90 mcg. L.-casei factor
- (11) 6 mgm. pantothenic acid, half as racemic calcium pantothenate and half as dextro calcium pantothenate
- (12) 300 mcg. para-aminobenzoic acid
- (13) 3 mgm. pyridoxine hydrochloride
- (14) 0.50 gram choline chloride as choline dihydrogen citrate

The subjects were in the age group 23 to 28, were free from organic defects, and carried on activities normally encountered in a university environment, i.e.,—classes, laboratory work, hikes, indoor athletics, and so forth. Throughout the experiment, measurements of physical and psychomotor performance were made, as well as other tests. Complete assays were made weekly on urine, feces, blood and food. The methods used in making these measurements and tests, as well as the overall clinical, physical and psychomotor findings, have been described in considerable detail elsewhere (3,4), and therefore are not repeated here. In the present report the following contrasts are made:

a The lowered urinary excretion levels of vitamins occurring soon after the experimental diet was introduced, in contrast to the relatively high and unchanged scores made in tests of physical and psychomotor performance.

b The high urinary excretion levels of vitamins occurring when the various supplements were received, in contrast to the then decreased scores made in tests of physical and psychomotor performance.

RESULTS. For the purposes of making these contrasts, physical performance scores on a bicycle ergometer<sup>3</sup> and psychomotor performance on a pursuit meter<sup>4</sup> have been chosen for discussion in relation to vitamin excretion levels found at corresponding times. Figure 1 demonstrates 1, the *slow* decline in the amount of work performed by the experimental subjects on the bicycle ergometer after the restricted intake was begun, with the *eventual* recovery occurring when supplementation was received, and 2, the *immediate* changes found in the urinary excretion levels of thiamine when the restricted intake was begun (table 2), with the *relatively rapid* return to normal excretion levels found when the thiamine supplements were received. It is apparent that for some 8 weeks following the introduction of the experimental diet, no change in work performance occurred, although the urinary excretion levels of thiamine, riboflavin, N<sup>1</sup>-methyl-nicotinamide, niacin and the "lesser-known" B-complex factors had been at very low levels through the greater part of that period. This situation is characteristic of that occurring for all 7 subjects during the early stages of the experiment and has been described in more detail elsewhere, under the heading, "Absence of rapid deterioration in moderately active young men on a restricted intake of B-complex vitamins and animal protein" (4). The foregoing title is in itself indicative of the completely divergent picture obtained between physical performance on the one hand and urinary excretion levels of nutrients on the other.

<sup>3</sup> Electrodynamic brake bicycle ergometer: a stationary bicycle frame arranged for a variable, electrically controlled resistance against rotation of the pedals. A double work period to exhaustion of the leg muscles was used, separated by a ten-minute rest on a cot. Measurements were made in terms of duration of each ride, and resting and post-exercise pulse rates.

<sup>4</sup> Pursuit meter: a miniature airplane device which records errors in correcting the positions of the indicator in three planes, involving separate use of both hands and the right foot.

Similar findings were obtained for riboflavin. These are shown in figure 2, in which the riboflavin excretion levels are plotted with performance on the pursuit meter. Here it is seen that not only was there no decrease in performance during the first 10 weeks, but that an *improvement* in performance was found on some occasions even in the presence of the *low* excretion levels.

Table 2 indicates the promptness with which the urinary excretion levels dropped following the introduction of the experimental diet that provided much lower levels of vitamin intake. On the occasion of the first 4-day collection of urine, at days 2 to 5 (or 9 to 12 days) inclusive, of the restricted intake, the decrease in vitamin levels in every instance had been both prompt and marked,

TABLE 2

*Comparison of 24-hour urine excretion on a normal diet with those after a few days on a restricted diet*

VITAMIN	DIET	SUBJECT NUMBERS				
		3	4	5	6	7
Thiamine, mcg.	Normal diet	150	270	230	270	320
	2-5 days restricted diet	50	70	80	70	120
Riboflavin, mcg.	Normal diet	1,020	740	620	940	820
	2-5 days restricted diet	360	310	220	290	130
N <sup>1</sup> -Methylnicotinamide, mgm.	Normal diet	4.4	2.4	3.1	4.2	3.8
	2-5 days restricted diet	2.2	2.2	1.4	2.8	2.5
Biotin, mcg.	Normal diet	29	38	36	29	43
	9-12 days restricted diet	17	14	16	16	19
Pantothenic acid, mgm.	Normal diet	2.9	2.8	3.7	3.3	3.0
	9-12 days restricted diet	2.0	1.8	2.0	2.0	2.1
Pyridoxine, mgm.	Normal diet	0.33	0.40	0.45	0.39	0.38
	2-5 days restricted diet	0.25	0.28	0.24	0.27	0.29

and the new values were in general only 50 per cent of those found one week previously. Data such as these are highly suggestive of the point that has been made in previous publications (1, 2), that urinary excretion levels are mainly reflections of immediately preceding intake levels, and may have but little relationship to tissue levels.

Upon supplementation, there occurred a steady rise in excretion levels, as is apparent from figures 1 and 2. Reference made again to the physical and psychomotor scores (same figures) however, shows that at numerous points when excretion levels of the vitamins approached or exceeded those found originally on the normal diet, improvement in performance had not progressed to the score levels originally found to be within the subject's capacity.

DISCUSSION. The claim might be made that physical and psychomotor performance tests are not as sensitive a measure of nutritional state as are biochemical findings, and that this is the chief reason for lack of correlation. This possibility is not denied, for it would seem likely that low excretion values of

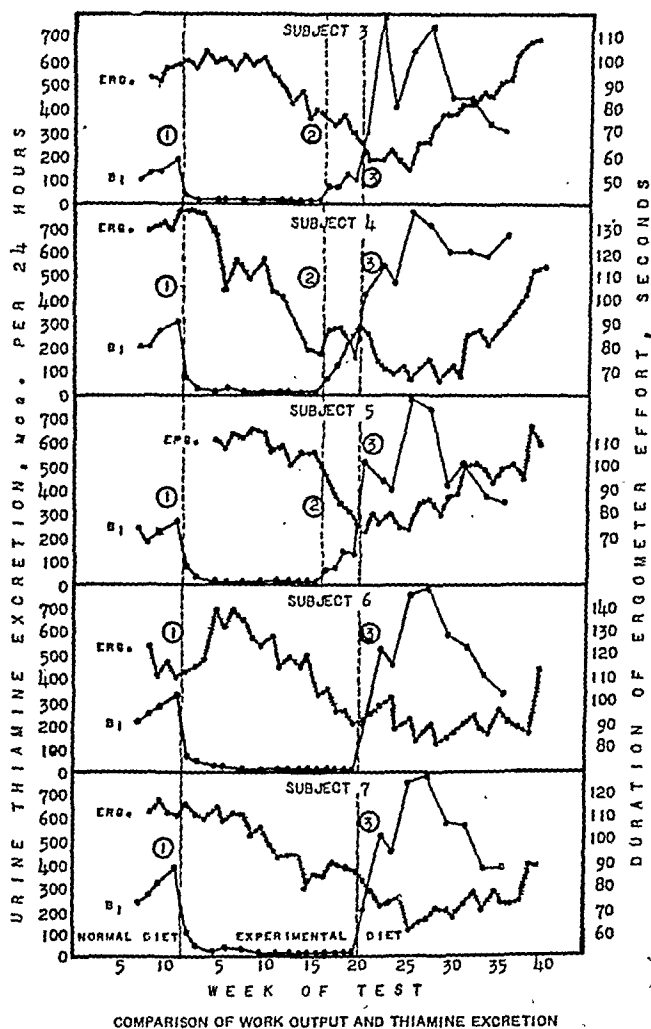
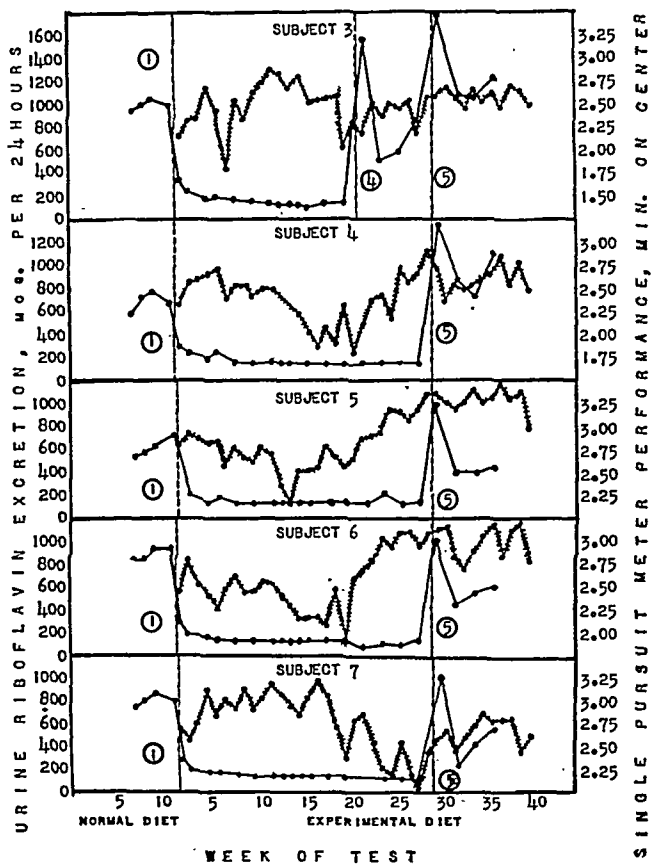


Fig. 1. Average 24-hour excretion of thiamine in the urine is shown by a solid line. Duration of effort on the bicycle ergometer is shown by a cross-hatched line. The broken vertical lines represent times at which certain dietary and supplementation changes were made. They are identified as follows:

- ① beginning of the experimental diet
- ② beginning of thiamine supplementation of 1.2 mgm. per day
- ③ two intravenous doses of 25 mgm. thiamine each. Other supplements were received thereafter; thiamine is used merely as one example.

long duration would be followed in the majority of instances by signs in the physical, psychomotor or clinical category. There is nevertheless a question to be raised concerning the importance that should be attached to single biochemical data which may appear at the moment to be below normal. This

question arises when it is recalled that decrease and then increase in performance, respectively, corresponded not so much to low and high urinary biochemical excretion levels, but rather to other indicative and objective information such as the presence or absence of subjective symptoms and clinical signs, and the investigators' careful judgment of the condition of the subjects. With these



COMPARISON OF PSYCHOMOTOR PERFORMANCE AND RIBOFLAVIN EXCRETION

Fig. 2. Average 24-hour excretion of riboflavin in the urine is shown by a solid line. Time on center in the single pursuit meter test is shown by a cross-hatched line. The broken vertical lines represent dietary and supplementation changes as follows:

- ① beginning of the experimental diet
- ② 10 mgm. riboflavin intravenously and beginning of 1.5 mgm. per day supplementation with riboflavin
- ③ beginning of one week's supplementation with 15 mgm. per day of riboflavin followed by 1.5 mgm. per day thereafter. Other supplements were received both prior and subsequent to the riboflavin; the latter is used here as one example.

facts in mind, it then becomes evident that the use of single or even a few biochemical determinations as self-indicative signs of nutritional state may be fraught with inaccuracy.

Evidence is presented in this report showing lack of correlation between urinary excretion levels and scores made on tests measuring physical and psycho-

motor performance. Performance on these tests is known to be adversely affected when nutritional deficiency is present. Similar evidence was obtained using other physical and psychomotor tests, and in the case of other vitamins, but these are not presented here, in order to permit brevity. Furthermore, it is not the intention of the authors to single out biochemical data exclusively in any manner that might appear to be derogatory. Rather, with these data available to us as a result of recent investigation, it is our purpose to present them as an example of the incorrect appraisal of nutritional state that may result from the use of any *one* type of data to the exclusion of other types. It is our belief that equally poor correlation could be found between certain types of purely clinical or dietary intake data on the one hand, and physical or psychomotor performance on the other. Our purpose is, as mentioned originally, to point out the need for interpreting with caution any data related to only one aspect of nutritional state, and to emphasize further the success in accurate nutritional appraisal that is made possible when all types of available data are used conjointly.

#### SUMMARY

The lack of correlation that may occur between a few urinary excretion levels of nutrients and measures of physical and psychomotor performance is shown. An even greater discrepancy could occur in the case of single biochemical readings. It is concluded that biochemical data, such as the urinary excretion levels of nutrients, are a valuable tool in nutritional appraisal especially when used in conjunction with, or corroboration of, other types of data, notably clinical, physical, psychomotor and dietary. If used alone, however, they may mislead, not only because they are influenced by previously attendant factors such as recent dietary intake, the normal amount of variation found among individuals, and possibly environmental factors, but also because there may be a considerable lag between changes in biochemical levels and corresponding changes in physical and psychomotor performance. Similar criticism can be made of other types of data if used singly in the appraisal of nutritional state, except where gross specific deficiency signs are involved.

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# STUDIES ON FROST-BITE WITH SPECIAL REFERENCE TO TREATMENT AND THE EFFECT ON MINUTE BLOOD VESSELS<sup>1</sup>

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The need for further research on the problem of frost-bite was indicated by the wide variety of procedures recently recommended for its treatment. At the time this investigation was begun, frost-bite was a major problem among the personnel of the Air Corps of the United States Army and this investigation was undertaken at the request of Colonel W. R. Lovelace, II, then of Wright Field. The purpose of the research was primarily to evaluate the different types of treatment that had been used for frost-bite with the hope that more effective procedures might be discovered. However, before the work had progressed far, it was found necessary to study the fundamental nature of the injury resulting from freezing.

Many hypotheses have been advanced to explain the reaction of tissues to cold. Lewis (15) stated that injury from frost-bite was due to liberation of a histamine-like substance by the tissues in the early stages. He described the effects of short periods of exposure to cold as a triple response characterized by local redness, followed by wheal and flare, and later by the formation of a blister. The liberated histamine-like substance acting on the vessels produced dilatation and increased permeability with subsequent edema and formation of the blister. Oxygen deficit resulted from obstruction of the circulation by the edema and by the increased tissue metabolism.

The occurrence of damage to blood vessels with vascular thrombosis and necrosis has been reported by Greene (7) and Lewis (16). Leriche (14) made arteriograms by injecting thorium dioxide sol (thorotrast) into the frost-bitten extremities in which gangrene developed. Complete obstruction and the projection of irregular nodules into the lumen of the vessels and the accompanying pathologic effects led him to deduce that thrombosis had taken place.

Vasospasm and hypertonia of the sympathetic nerves of the arteries after frost-bite have been noted by several writers. Ducuing (5) reported apparent vasospasm and hypertonia of the sympathetic nerves in the limbs of human beings exposed to frost-bite. Burdenko (2) supported this same idea. In addition European workers have presented evidence that vascular stasis occurs after the initial reaction to frost-bite.

There are few reported observations on the histopathology of frost-bite.

<sup>1</sup> Abridgment of thesis submitted by Dr. Quintanilla to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Physical Medicine.

Smith, Ritchie and Dawson (19) in 1915 after microscopic examination of tissues stated that the chief effect of cold was on the blood vessels. They reported dilatation of the lumen of the vessels with deposition of fibrin, swelling of the endothelium of the intima and vacuolation in the muscle fibers of the media. The lesion in the nerves consisted of a swelling of the axis-cylinders which swelling appeared to be a result of the general edema. Other studies of the pathologic effects of frost-bite have been made on specimens obtained at amputations performed many days after freezing.

Bigelow (1) reviewed all the methods of treatment that have been used in cases of frost-bite and the general principles underlying them. He called attention to the good and sometimes excellent results obtained by many investigators with very different types of treatment. Duction reported slow but complete recovery when massage of the tissues above those that were frozen was carried out in a cold room. Greene (8) described the value of a temperature of 2° or 3°C. maintained for several days to a week applied locally to the part frozen. He used a therapeutic refrigerator in his work. Two Italian workers, Uffreduzzi (20) and Cignolini (3) reported the successful use of short wave diathermy. They thought that it helped to restore the circulation and promoted the final delimitation of the gangrene. Passive vascular exercise has proved to be beneficial according to Herrmann's (9) work in twenty-three cases of frost-bitten extremities in which he used the pavex machine. According to Bigelow the Canadian army has suggested immersion of the unaffected limb in hot water in order to produce reflex vasodilatation in the frozen extremity. Bigelow pointed out that the use of vasodilator substances has never been discussed in the literature and that the value of heparin has never been completely investigated. Arteriotomy and vitamin therapy have been advocated (1) and block of the sympathetic nerves and sympathectomy have been used. Leriche (13) reported a series of thirty-nine cases in which the lumbar or stellate ganglia were infiltrated with an anesthetic agent. Immediate relief of pain resulted and gangrene did not develop in any cases in which this treatment was given early. Burdenko recommended the same kind of treatment. Davis and his associates (4) used the sympathetic block and injection of stellate ganglion with procaine hydrochloride and concluded that acute dilatation of the peripheral capillary bed could be obtained if there had not been permanent injury to the capillary walls or thrombosis at the junction of arterioles and capillaries.

It is noteworthy that the majority of the methods of treating frost-bite were designed to increase the blood flow to the affected part at an early stage. It is striking that despite the numerous types of treatment used, no single procedure has prevailed over the others and as far as we know, no classification has been made in which the various degrees of injury resulting from freezing are distinguished.

One of the great difficulties in evaluation of various types of treatment of frost-bite is the lack of a method of determining, immediately after freezing, the amount of damage to be expected in the affected tissues. This lack is important because it is during the time, immediately following thawing before edema

develops that the treatment, if there is to be any, must be instituted. Furthermore, it often happens that even after the development of edema, the degree of injury is difficult to assess. As might be expected, the deeper the freezing the greater the resulting damage, consequently the prognosis is better in those cases in which only the skin has been affected than in those in which the underlying tissues as well as the skin are involved. The depth of freezing depends on the time of exposure and the degree of temperature, varying, of course, with the part of the body exposed. There is, for example, a difference in the degree of freezing of the fingers and the cheek if both are exposed to the same temperature for the same length of time. Many reasons have been given to explain this difference. Even in experimental work in which conditions can be established more nearly as desired, the results vary because of anatomic and individual reactions to freezing (17). Some animals are able to maintain the circulation in an extremity under conditions that cause the extremities of other animals to freeze solid.

**METHODS.** The most important consideration in performing experiments on frost-bite is the selection of a method that insures uniformity in the application of the cold as well as constancy in the degree of freezing of the tissues in successive experiments.

After a series of preliminary tests it was concluded that a mixture of absolute alcohol and carbon dioxide ice was the most satisfactory means of freezing the tissues. The temperature of the mixture was determined easily by a toluol (Cenco) thermometer capable of registering  $-100^{\circ}\text{C}$ . Initially, the temperature of the alcohol was kept near the desired point by adding or withdrawing pieces of carbon dioxide ice of the proper size. Later, a container made of wire mesh was used for placing the carbon dioxide ice in alcohol. This made possible the maintenance of the temperature within  $0.5^{\circ}\text{C}$ .

Rabbits were used as the experimental animals. The forefeet and legs were chosen for freezing. Routinely the animals were anesthetized with pentobarbital sodium (25 mgm. per kgm. of body weight) but a few were not given the anesthetic agent. Before freezing, the foot and leg to be frozen were clipped closely. The feet of the control and the treated animals were submerged in the freezing solution simultaneously in the same container. The extremities were thrust into the freezing mixture from one-third to one-half way to the elbow. Freezing was done at temperatures ranging from  $-12^{\circ}$  to  $-50^{\circ}\text{C}$ . The time varied from one to ninety minutes. The thermometer was kept in motion and under constant observation. If some form of treatment was used, it was usually begun immediately after the extremity was removed from the freezing mixture. It required from ten to twenty minutes for the limbs to thaw at room temperature ( $26^{\circ}\text{C}$ .) after they were frozen at the lowest temperatures. Unless otherwise stated this method of thawing was used.

The methods of treatment used in this and other studies may be classified as follows: 1, thermal; 2, mechanical, and 3, chemical. Thermal methods consisted of submersion of the frost-bitten part in warm or cold water or application of cold or heat (radiant heat and diathermy) to the frozen part. The

mechanical methods used were *a*, application of a tourniquet; *b*, bandaging (Ace bandage) or application of a plaster cast to the frozen extremity, or *c*, massage of tissues immediately adjacent to the frozen part. Chemical treatment consisted of administration of such vasodilating drugs as histamine and papaverine, administration of hypertonic solution of glucose, blocking the nerves with various local anesthetic agents, and use of heparin to prevent coagulation of blood in the vessels of the frozen extremity.

For the study of the effect of freezing on the minute blood vessels, a different method was necessary. This will be explained with the results of that part of the investigation.

**RESULTS.** *Freezing at  $-20^{\circ}$  to  $-50^{\circ}$  C. for one to five minutes.* In order to gain knowledge of the temporary and permanent effects of mild and severe frost-bite, a series of observations was made on the feet of rabbits frozen for various lengths of time at different temperatures. Freezing was done at  $-20^{\circ}$  to  $-50^{\circ}$  C. with the time of exposure ranging from one to five minutes. Regardless of whether the feet were exposed for three to five minutes at the higher temperatures or for one to three minutes at the lower temperatures they were usually white and hard on removal from the freezing solution. The degree or depth of freezing was impossible to determine from the gross appearance of the feet immediately after freezing. The appearance of all was similar. Had it not been for the fact that we knew the temperature at which the feet were frozen, we would not have anticipated the subsequent changes.

Inspection of the feet one week after freezing disclosed markedly different results. The animals whose feet were frozen at the higher temperatures were not greatly inconvenienced since they had lost, at most, only small patches of superficial tissue whereas those whose feet were frozen for three minutes at the lowest temperatures ( $-40^{\circ}$  and  $-50^{\circ}$  C.) had lost the entire foot and part of the leg. With certain exceptions to be described the severity of the freezing for a given length of time appeared to be roughly proportional to the degree of cold to which the feet were subjected. The results following immersion of the feet for three minutes at  $-40^{\circ}$  and  $-50^{\circ}$  C. were similar. The injury at these temperatures apparently was maximal (table 1). An attempt to treat the feet of the animals of this series was not made. Not all the feet exposed to the same temperature for an equal length of time exhibited exactly the same result. There was a slight difference among them in the amount of swelling, the final outcome depending perhaps on anatomic and individual variations which were difficult to appraise. It seems likely that the most important factor in these differences was the circulation since some animals, as shown in table 2, were able to withstand temperatures that caused the feet of other animals to freeze.

*Various methods of treatment.* Regardless of the method of treatment devised for the frozen feet of rabbits, the outcome was entirely negative. The untreated controls almost without exception did just as well as the treated animals. Placing the frozen feet in cold water or in water at body temperature for as long as five hours after freezing was without benefit. Applying occlusive tourniquets to limbs immediately after freezing and thawing them in water at body tem-

perature or slowly in ice water was not better than allowing the feet to thaw at room temperature. Placing an occlusive tourniquet on the frozen limbs before or after freezing and releasing the pressure gradually so that a sudden inrush of blood could not occur after thawing were not of value.

TABLE 1

*Results of freezing the feet of rabbits at different temperatures for one to five minutes.  
No treatment given*

NUM- BER OF RAB- BITS	FREEZ- ING TEMPER- ATURE	DURA- TION OF EXPO- SURE	RESULTS			COMMENT
			2 hours after removal	1 week later	Function	
3	-20	3	Slight swelling mainly in the toes	Complete recovery.	Good	
4	-20	5	Swelling of whole foot in all with more distention of skin in 1 rabbit	No loss of tissues	Good	Thickening of the toes in 2 rabbits
3	-30	3	Swelling of limb to elbow	Spots of dry skin on dorsal part of foot in 2 rabbits	Good	Residual thickening of the foot in 2 rabbits. Thickening of toes only in the other
5	-40	1	Slight swelling mainly in toes; swelling of whole foot in 1 rabbit	Recovery	Good	Thickening of the toes in 1 animal
3	-40	3	Swelling of whole limb with distention of the skin of foot. Oozing from skin in 1 rabbit	Dry gangrene	Bad	Permanent loss of control of foot
4	-50	3	Swelling of whole limb with distention of skin and oozing from skin	1 rabbit died 4 days after foot was frozen. All feet frozen destroyed by dry gangrene	Bad	Loss of control of foot after freezing

Wrapping the limb frozen at  $-40^{\circ}\text{C}$ . for three minutes before or after thawing with an Ace bandage or applying to it a plaster cast (6) did not result in lasting benefit to the animals receiving this treatment over their untreated controls. This was true whether the wrappings or casts were left on for one day or as many as thirteen days. It should be stated that the Ace bandage prevented swelling during the time it was in place, but the limb became edematous soon after the bandage was removed, except in those instances when it was left on the limb for

TABLE 2

*Results of experiments to determine the effect of different doses of heparin on the feet of rabbits frozen for various lengths of time and at different temperatures*

RABBIT	DURATION OF EXPOSURE	TEMPERATURE	TREATMENT	RESULTS
	<i>min.</i>	<i>°C.</i>		
1	3	-30--36	1,000 units of heparin before freezing	Frozen portion of extremity lost
2	3	-30--36	None; control on rabbit 1	Same as rabbit 1
3	3	-43--47	1,000 units of heparin before freezing	Frozen portion of extremity lost
4	3	-43--47	None; control on rabbit 3	Same as rabbit 3
5	3	-23--28	1,000 units of heparin before freezing	Almost complete recovery
6	3	-23--28	None; control on rabbit 5	Same as rabbit 5
7	3	-25--27	1,000 units of heparin before freezing	Frozen portion of extremity lost
8	3	-25--27	None; control on rabbit 7	Lost 2 toenails
9	15	-25	None	Frozen portion of extremity lost
10	3	-25	None	Recovery
11	15	-25	None	Frozen portion of extremity lost
12	3	-25	None	Recovery
13	15	-25	None	Frozen portion of extremity lost
14	3	-25	None	Recovery
15	90	-20	1,000 units of heparin before freezing	Frozen portion of extremity lost
16	90	-20	None; control on rabbit 15	Same as rabbit 15
17	80	-20	1,000 units of heparin before freezing	Frozen portion of extremity lost
18	80	-20	None; control on rabbit 17	Same as rabbit 17
19	90	-20	None	Frozen portion of extremity lost
20	90	-20	None	Same as rabbit 19
21	60	-12	1,000 units of heparin before freezing	Lost foot
22	60	-12	None; control on rabbit 21	Foot did not freeze; complete recovery
23	60	-12	1,000 units of heparin before freezing	Lost 3 toes
24	60	-12	No treatment; control on rabbit 23	Died before completion of observation
25	30	-15	1,000 units of heparin before freezing	Frozen portion of extremity lost
26	30	-15	None; control on rabbit 25	Foot did not freeze. Recovered fully
27	20	-15	1,000 units of heparin before freezing	Frozen portion of extremity lost
28	20	-15	None; control on rabbit 27	Same as rabbit 27

TABLE 2—*Concluded*

RABBIT	DURATION OF EXPOSURE	TEMPERATURE	TREATMENT	RESULTS
	<i>min.</i>	<i>°C.</i>		
29	20	-20	Tourniquet applied for 3 min.; 1,000 units of heparin	Frozen portion of extremity lost
30	20	-20	Tourniquet applied for 3 min. but no heparin given	Same as rabbit 29
31	20	-20	Tourniquet applied for 3 min.	Foot frozen hard; frozen por- tion of extremity lost
32	20	-20	Same as rabbit 31; in addi- tion heparin given for 36 hrs.*	Same as rabbit 31
33	20	-20	Tourniquet applied for 3 min.	Foot frozen hard; frozen por- tion of extremity lost
34	20	-20	Same as rabbit 33 but in addi- tion heparin given for 36 hrs.*	Same as rabbit 33
35	20	-20	Tourniquet applied for 3 min.	Foot frozen hard; frozen por- tion of extremity lost
36	20	-20	Same as rabbit 35 but in addi- tion heparin given for 36 hrs.*	Same as rabbit 35

\* 1,000 units of heparin were given intravenously before freezing the foot and 500 units every 3 hours thereafter for 36 hours.

as long as eight to thirteen days. When it was removed after eight to thirteen days, swelling did not occur, but the limb was of little immediate use to the animal and eventually was lost entirely.

The possibility of thrombosis of the vessels of the frozen tissues has been emphasized by many authors. An injection of a solution of heparin in normal saline solution was given intravenously to the rabbits, alone or in combination with other forms of treatment. With this procedure the blood of the animal was maintained noncoagulable during as well as after the time when the blood was allowed to return to the frozen foot. Hypertonic solutions of glucose also were given intravenously in certain instances in an attempt to diminish the loss of fluid into the tissues. The results of the experiments in which the cuff or tourniquet was employed alone or in combination with intravenous administration of heparin or other procedures were not significantly different from those seen in the controls that were not treated at all.

In one series of experiments the rabbits' feet, immediately after being frozen, were wrapped in a rubber sheet to keep them dry. They were then placed in a bag filled with cracked ice. Temperature in the bag was between 4° and 5°C. The temperature at which the feet were frozen varied. Some were frozen at -42°C. for three minutes, others at -45°C. for one minute and the feet of three rabbits at -48°C. for two minutes. Different results were obtained according to the temperature and duration of immersion. Packing in ice did not prevent the

formation of edema in the frozen extremity. Swelling was present after keeping the frozen limb packed in ice for one hour and a similar result followed the use of the same procedure for six hours.

After the experiments just described were completed, the papers of Lange, Boyd (11) and Loewe (12) appeared and a number of confidential reports were received in which heparin was said to be of great benefit in the treatment of frost-bite. Since we had given only enough heparin to keep the blood noncoagulable for a few hours, and others had given the drug for as long as three days, it seemed necessary to make further observations.

*Additional studies on treatment with heparin* (table 2). In the experiments now to be described, the feet of some rabbits were frozen for short periods at low temperatures, and those of others were frozen for long periods of time at higher temperatures. Heparin was given intravenously before freezing. In certain experiments only one injection of 1,000 units of heparin was given while in others the blood was kept noncoagulable for thirty-six hours by giving 500 units of heparin every three hours following freezing.

Feet frozen for three minutes at temperatures ranging from  $-43^{\circ}$  to  $-47^{\circ}\text{C}$ . and at temperatures ranging from  $-30^{\circ}$  to  $-36^{\circ}\text{C}$ . were destroyed even though the blood remained noncoagulable for three hours by injection of 1,000 units of heparin immediately before freezing (rabbits 1 to 4, table 2). The effects were variable when the feet were frozen for three minutes at  $-23^{\circ}$  to  $-28^{\circ}\text{C}$ . and  $-25^{\circ}$  to  $-27^{\circ}\text{C}$ . Out of four animals (rabbits 5 to 8, table 2) three almost completely recovered while the fourth animal (rabbit 7) that had been given heparin lost the frozen portion of the extremity.

In order to determine the effect of freezing at a given temperature for different periods of time a series of observations was made on three pairs of animals (rabbits 9 to 14, table 2). The foot of one animal in each pair was frozen for three minutes while the foot of the other in the pair was frozen for fifteen minutes at  $-25^{\circ}\text{C}$ . In every instance the foot that was kept in the freezing mixture for three minutes fully recovered while the one that was held in the freezing mixture for fifteen minutes was destroyed. The feet of two rabbits (rabbits 17 and 18) frozen at  $-20^{\circ}\text{C}$ . for eighty minutes and the feet of two other pairs (rabbits 15, 16, 19 and 20) frozen at  $-20^{\circ}\text{C}$ . for ninety minutes were lost. Three of the animals whose feet were frozen at  $-20^{\circ}\text{C}$ . had been given 1,000 units of heparin before the foot was submerged in the freezing mixture. Two of the four animals (rabbits 21 to 24) whose feet were frozen at  $-12^{\circ}$  for sixty minutes were given 1,000 units of heparin before freezing. It so happened that one of these two animals lost the frost-bitten foot and the other lost three toes. In contrast the foot of one of the control animals (rabbit 22) did not freeze at all and consequently was uninjured by the exposure. Another control animal died before the observations were completed. A similar result was obtained when the feet of two rabbits (rabbits 25 and 26) were submerged for thirty minutes in a freezing mixture at  $-15^{\circ}\text{C}$ . The rabbit that had received the heparin lost the extremity while the foot of the control animal failed to freeze and consequently fully recovered. Another pair of rabbits was used and

the feet were submerged for twenty minutes at a temperature of  $-15^{\circ}\text{C}$ . The one that received the injection of 1,000 units of heparin lost its foot and so did the control animal.

From these observations it became evident that considerable variability may be expected in the results of experiments done as described since the foot of one animal was frozen solid when submerged at a given temperature and the foot of another animal failed to freeze at all when placed for the same length of time in the same solution. In order to control the factor of the circulation to the limb during the initial freezing an occlusive tourniquet was placed on the limb for three minutes. Four pairs of rabbits were used in this series of observations (rabbits 29 to 36). One animal of each pair was given 1,000 units of heparin before the foot was frozen. The blood of three rabbits that received the heparin was kept noncoagulable for thirty-six hours by giving 500 units of heparin intravenously every three hours. By use of a tourniquet we were able to freeze the extremities of each pair of rabbits within three minutes after they were submerged in the freezing mixture. The extremities were submerged for twenty minutes at  $-20^{\circ}\text{C}$ . In the animals treated with heparin and those that were not, the result was the same; namely, the frozen portion of the extremity was completely lost. The results of the freezing were evaluated daily by a colleague who did not know which animals were controls and which had been treated. In every instance in the early stages the feet of the animals that had received the heparin appeared in much worse condition than the feet of the controls.

After reviewing all of the data obtained, we came to the conclusion that any treatment used in the presence of a severe tissue damage was ineffectual.

*Studies of small vessels and the type of lesion.* An important question to answer was: what is the nature of the lesion produced in the tissues exposed to freezing? It is well known that at the time of freezing there is vasoconstriction indicated by the whitish appearance of the affected region. This vasoconstriction is followed after thawing by vasodilatation as evidenced by redness and warmth of the frozen tissues. When thawing is accomplished, the swelling starts, which means that soon after the circulation is re-established, the damage becomes evident. It is apparent that the primary effect of cold is on the blood vessels, especially on the minute vessels whose walls are extremely thin and are, therefore, presumably more vulnerable than the larger ones.

In an attempt to learn what happens to the smaller vessels and how they behave after freezing, observations were made on blood vessels that had grown into a transparent chamber inserted into the ears of rabbits. The window used was Florey's modification of the Clark window. A window which was completely filled with vessels was studied under the microscope. A small piece of solid carbon dioxide was placed in contact with the sheet of mica covering the vessels. The piece of carbon dioxide was maintained in place for thirty seconds. After removal of the dry ice, it was found that all the vessels in the window had disappeared as a result of the freezing. After thawing, however, all the vessels opened and the blood flowed freely even in the spot that was frozen. The vessels at that particular time showed no apparent damage and their appearance was

not different from that which was observed before freezing. Anyone looking at the window at this time would not suspect that the tissues had been frozen.

From five to ten minutes after thawing the blood began to move more slowly in the vessels of the previously frozen region and the blood cells began to be concentrated until the engorged vessels were completely packed with them. By this time the blood flow had ceased in the region that had been frozen; however, at its edges there was still a to-and-fro movement of blood cells, especially in the arterioles. The blood cells appeared to be clinging together forming a mass as if they had lost their normal cellular outline; but if a slight pressure was applied at the window, a part of the mass could be seen to break up and individual cells flowed away and entered into the circulation. Then a new stream of blood came into the vessels and filled them again as before. No vessel affected by the freezing ruptured as it might have been expected to do at the time when the circulation was re-established and few blood cells escaped to the surrounding tissues. Five minutes after they were packed with cells the previously frozen vessels could still contract as well as the normal ones if the rabbit was frightened or pinched. Two hours later the mass of red cells in the vessels remained the same except that the color of the blood had changed from a red to a pink color.

In the following days disintegration of the frost-bitten region occurred and by the end of the fourth day, only a few brown and yellow spots were left. If the spot frozen was not too large and if good circulation surrounded it, new vessels developed. About one week after freezing, the area that had been frozen was filled with newly formed vessels.

In order to eliminate any possibility of coagulation, 50 units of heparin per kilogram of body weight (Connaught, liquid heparin 1,000 units per cubic centimeter) was given intravenously to a rabbit ten minutes before the experiment and the injection was repeated immediately before freezing. A part of the vessels in the window of the rabbit's ear was frozen. The length of time used for freezing was thirty seconds as before. The phenomenon already described was observed in this case. Coagulation did not take place in the injured vessels, nor was a thrombus formed at any arteriolar capillary junction. The obvious fact is that, as an immediate result of freezing, the minute vessels became packed with blood cells thus isolating them from the general circulation. The after-effects of freezing should be attributed in part to stasis.

The simplest explanation of the stasis observed after freezing is that the increased permeability of the damaged walls of the vessels allows the plasma of the blood to escape into the tissues thus leaving the blood cells concentrated in the vessels. This explanation is supported by the fact that stasis did not occur immediately after freezing the tissue. A certain period of time passed before the blood flow became sluggish and the corpuscles became concentrated. The presence of stasis after freezing was described by Rotnes and Kreyberg (18) who studied the phenomenon in the ears of rabbits. Small regions were frozen for different lengths of time in the ears of a rabbit which had previously been given an injection of vital stain (lithium carmine). An intracardial injection of India ink was given twenty-four hours later. The injection was given to a

control series fifteen minutes after freezing. The animals were killed and the ears were fixed in formalin and studied microscopically. These investigators concluded that although a minor injury produced dilatation of the vessels with exudation, shown by the staining of the tissues, a severe injury caused an exudation of all the fluid of the blood, leaving the vessels blocked by a column of blood cells. They based their opinion that stasis was present on the fact that the India ink failed to fill the affected vessels. Most American and English writers have not considered stasis as a fundamental lesion in frost-bite but have emphasized the formation of thrombi. Rotnes and Kreyberg's work may not have impressed them because of the fact that these workers did not exclude the possibility of formation of a thrombus.

The stasis following freezing is not a temporary pooling of the blood produced by the diminution or arrest of the blood flow but is rather a concentration of the blood cells caused by the loss of fluid from the injured vessels. The presence of stasis in the previously frozen vessels in the transparent chamber in the ears of a rabbit whose blood has been rendered incoagulable makes untenable the belief that the stasis resulting from freezing is owing to coagulation of blood. The primary effect appears to be injury of the walls of the vessels which results in increased permeability as previously reported by Lake (10). The injury usually does not disrupt the wall of the blood vessels. Gross hemorrhage into the injured tissue was not usually seen. Therefore it avails little to increase the blood flow in the frozen region in an attempt to wash out the packed cells because as long as the damage of the wall of the vessels is present, the result will be the same, that is, the plasma will pass through the walls of the vessels. Quicker delimitation of the damage cannot be obtained by increasing the rate of flow; the production of more edema would compress the surrounding tissues and make the chances of recovery poor.

#### SUMMARY AND CONCLUSIONS

Because of the low temperatures at high altitudes, frost-bite has been a serious problem among aviators. Frost-bite among ground troops required to campaign during winter has not been uncommon. The lack of adequate information as to the nature of the lesion caused by freezing has made difficult the development of a rational therapeutic procedure. Consequently the treatment employed has varied from hot applications to placing the frozen part in ice packs. The need for more experimental work was apparent.

The first problem was the development of a reliable method of freezing. The one finally adopted consisted of immersing the anterior extremities of rabbits in absolute alcohol to which pieces of carbon dioxide ice were added until the desired temperature was obtained.

The extremity was frozen at various temperatures for different lengths of time. Immersion of the foot in the freezing mixture for three minutes at a temperature of  $-40^{\circ}\text{C}$ . usually resulted in total destruction of the frostbitten part. A similar result followed freezing the extremity at  $-20^{\circ}\text{C}$ . for twenty minutes.

Many of the methods of treatment employed by previous workers as well as

those used by the medical corps of the army were studied. Briefly, the results were discouraging. It was impossible by inspection to distinguish between extremities that were frozen beyond hope of recovery (at  $-40^{\circ}$  to  $-50^{\circ}\text{C}$ . for 3 min.) and those that were less seriously frozen ( $-20^{\circ}$  to  $-30^{\circ}\text{C}$ . for 3 min.).

Without a knowledge of the severity of the injury resulting from frost-bite, an evaluation of the effectiveness of a given treatment cannot be made. Therapeutic measures will give good results in mild cases but poor results in severe cases of frost-bite. All of the recommended methods of treatment of frost-bite were useless when applied to the severely frozen feet of rabbits. The controls recovered quite as well as the treated animals. Whether the frost-bite was severe or mild, all of the therapeutic measures used were no better than none at all.

It has long been thought that frost-bite results in coagulation of the blood in the smaller vessels and eventual anoxia which causes further destruction of the frost-bitten tissue. To investigate this question we made use of the Florey modification of the Clark transparent chamber inserted into the ears of rabbits. Pieces of carbon dioxide ice were applied directly to the window for thirty seconds. The vessels that had grown into the chamber were completely frozen. Immediately after thawing, the vessels appeared normal in every respect. However, five or ten minutes later it was apparent that serious injury had been done. The vessels dilated widely and became filled with red and white blood cells. The permeability of the walls of the vessels increased, the plasma was drained from the vessels and the formed elements were left concentrated in the vessels in conglomerate masses. These masses were not owing to coagulation since an identical picture was seen in the vessels of animals whose blood had been made noncoagulable by injection of heparin.

A number of papers have appeared recently in which it has been claimed that the frozen feet of rabbits have been saved by giving heparin for three days. In our hands prolonged administration of heparin to rabbits whose extremities were frozen did not save the feet of the animals.

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# THE EFFECT OF PILOCARPINE, MECHOLYL, ATROPINE AND ALCOHOL ON THE GASTRIC POTENTIAL AND THE SECRETION OF HYDROCHLORIC ACID<sup>1</sup>

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A series of investigations has been undertaken in an attempt to find out whether electrical energy furnishes the energy necessary for the production of the osmotic work involved in the formation of HCl by the stomach. One approach to this problem is the study of the relationship between the rate of secretion of HCl and the potential difference across the stomach wall (referred to in this paper as potential or overall potential).

Previously published results have demonstrated that, under certain experimental conditions, there is a relationship between these two characteristics. It has been shown that after histamine stimulation the potential decreases and that this decrease is associated with the onset of secretion (9). It was also shown that after the potential reaches a relatively constant level the secretory rate may increase considerably (9). It was found that in a stomach stimulated by histamine the injection of thiocyanate results in a reduction of the secretory rate to zero and a concomitant increase in the potential to approximately its resting level (12). Application of relatively concentrated HCl solutions and subsequent replacement with saline (9) or the passage of an electric current from mucosa to serosa (10) results in a lowering of the potential of the secreting stomach and a decrease in the secretory rate.

It is possible to design an almost unlimited number of additional experiments that might be expected to throw further light on this relationship. Of the possible additional experiments, there are certain ones, the results of which taken in conjunction with previously published data might be expected to give a relatively complete picture of this relationship under the conditions of these experiments. Specifically the experiments presented in the present paper attempt to determine the relationship between secretion and potential *a*, when certain parasympathomimetic drugs are used as gastric stimulants in place of histamine; *b*, when atropine is administered after the initiation of secretion by these drugs, and *c*, when ethyl alcohol is used to lower the potential of the secreting stomach.

**METHODS.** The technique for measuring gastric secretion and potential described in a previous paper (9) was employed. With this technique a portion of the stomach along the greater curvature is placed between a ring of lucite and a lucite chamber. The ring of lucite has an oblique cut in it so that the blood vessels could be slipped inside it, thereby insuring an intact blood supply to the portion of the stomach in the chamber. The mucosal side of the stomach

<sup>1</sup> This investigation was aided by a grant from the Joseph E. Seagrams and Sons Company.

was oriented toward the chamber and the chamber was filled with normal saline unless otherwise specified. The secretory rate was determined every ten minutes by draining and flushing the chamber with saline. The total volume of each ten-minute sample was 100 ml. The pH and titratable acidity were determined on each sample in most of the experiments. In some experiments only the pH was measured. A non-polarizable electrode made contact with the fluid in the chamber and another similar electrode was placed in contact with the serosa. The potential difference was measured with a potentiometer.

Dogs were used in these experiments and sodium amytal (70 to 90 mgm. per kgm. subcutaneously) was used as the anesthetic agent.

It was found in all of the following experiments that the potential had the same orientation as that found in previous experiments, i.e., the serosal electrode was positive to the mucosal electrode in the external circuit.

**RESULTS.** *Effect of pilocarpine, mecholyl, and atropine on gastric secretion and potential.* As was pointed out above, histamine was used to stimulate gastric secretion in all of the previously reported experiments. The question arises as to whether the same relationship exists between the secretory rate and potential when gastric stimulants other than histamine are used. In an attempt to answer this question several other methods of eliciting gastric secretion were tried.

Figure 1C represents an experiment in which 1 mgm. of mecholyl (acetyl-beta-methylcholine chloride) was administered subcutaneously every ten minutes. In this experiment it can be seen that the administration of mecholyl was followed by a decrease in the potential and that this decrease was associated with the onset of secretion. It should be noted that the secretory rate increased to a maximum and then decreased in spite of the fact that the administration of mecholyl was continued throughout the experiment. This type of secretory response is similar to that found by Gray and Ivy (3) when mecholyl was administered to unanesthetized dogs with total stomach pouches. In contrast to this secretory response to mecholyl the use of histamine as the stimulant results in a secretory rate that can be maintained at a high level indefinitely, unless the state of the dog deteriorates markedly or some other experimental procedure is interposed (3, 12).

The resting level of the potential in this experiment (around 50 mv.) was lower than that found in previously reported experiments (12). Most of the experiments reported here were performed in the summer of 1946, and during that period a fairly large number of the dogs used had relatively low resting gastric potentials. It can be seen in figure 1C that the potential dropped to 32 mv. and then gradually increased to around 40 mv. This type of response has also been seen following histamine stimulation (compare fig. 1C with fig. 2B). It is obvious from examination of figure 1C that the secretory rate can vary over a wide range while the potential shows relatively little change.

It can also be seen in figure 1C that administration of atropine (after the secretory rate had fallen to a relatively low value) is followed by a rise of the potential to a level above the pre-secretion level and a decrease in the secretory rate to zero. In this experiment the secretory rate reached an extraordinarily



an error. In this experiment the glass electrode was not calibrated before and after the determination of the pH of each sample, as was done in some of the previously reported work (10), but was calibrated only once for the determination of the pH of all of the samples in this experiment. An error in the pH of 0.03 could account for the discrepancy between the secretory rate calculated from pH data and that calculated from titration data in the sample at the height of the secretory response.

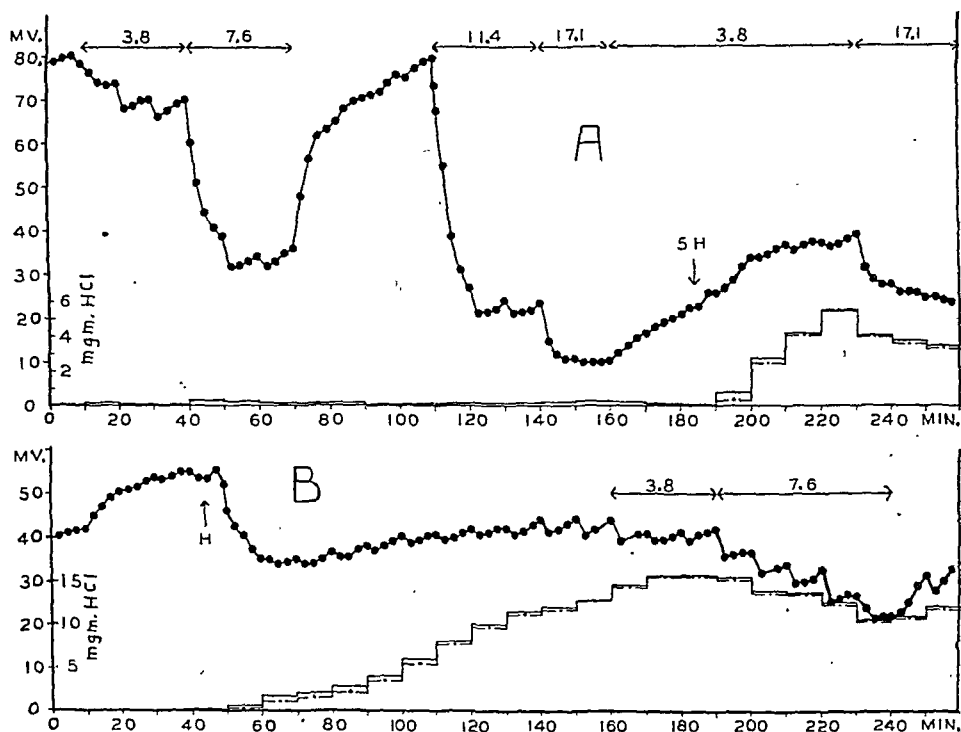


Fig. 2A and B. Relationship between secretory rate and potential difference. Period during which alcohol-saline solutions were used in the chamber in place of 0.9 per cent saline solutions indicated by horizontal lines with numbers above them. The numbers represent the concentration of alcohol (on a volume basis) in the alcohol-saline solutions. Secretory rates given in milligram of HCl secreted per ten minutes. Same conventions for secretory rates as in figure 1C. In figure 2A, a single dose of 5 mgm. of histamine diphosphate given at time indicated by arrow labeled 5H. In figure 2B, at time indicated by arrow labeled H, 2 mgm. of histamine diphosphate were given subcutaneously. Following this  $\frac{1}{2}$  mgm. of histamine diphosphate was given every ten minutes for the duration of the experiment.

A total of five dogs was used in the mecholyl experiments. In two of these dogs (one of which is represented by fig. 1C) a relatively high secretory rate was obtained and the potential curves were similar to those following histamine stimulation. In one of the dogs there was no evidence of secretion in spite of the fact that approximately the same dose of mecholyl was given as that in the dog represented in figure 1C. Since Gray and Ivy (3) found that large doses of mecholyl tend to be less effective than smaller doses, the next two dogs

were given small doses at ten-minute intervals and the dose was gradually increased. In one dog the maximum secretory rate obtained with this method was only 0.8 mgm. of HCl per ten minutes. In this experiment the potential dropped from a resting level of 62 mv. to a level of 43 mv., and the potential curve was quite similar to the response following histamine stimulation.

In the other dog the only evidence of secretion was a drop in the pH of the 100 cc. samples of from 6.0 to 5.5. This experiment is represented by figure 1B. The dose of mecholyl was 0.1 mgm. per ten minutes (subcutaneously) at the start (not shown in fig.) and was increased to 0.8 mgm. per ten minutes. The pH of the 100 ml. samples remained between 6.0 to 6.3, and the potential remained between 63 and 66 mv. for a period of 110 minutes prior to the drop in pH shown in figure 1B. The potential dropped, as can be seen in figure 1B, from 66 mv. to approximately 58 mv., while the pH dropped to 5.5. The administration of atropine resulted in an increase of the potential to 64 mv. and an increase of the pH to approximately 6.0. Further administration of much larger doses of mecholyl did not result in a decrease in the pH. It is apparent that a decrease in the pH from 6.0 to 5.5 represents an extremely small rate of secretion of hydrogen ions. However, in all of the previously published experiments a spontaneous decrease of the pH of this amount was never observed.

Seven experiments were performed with pilocarpine hydrochloride. In three of these experiments there was a decrease of the potential associated with the onset of secretion very similar to the curves obtained after histamine stimulation (potential leveled off in neighborhood of 40 mv.). The maximum secretory rate in all three experiments was around 2 mgm. of HCl per ten minutes. In all of these pilocarpine experiments the secretory rate reached a maximum and then decreased. It was not possible to maintain a relatively constant secretory rate by the regular administration of pilocarpine.

In two of the pilocarpine experiments no secretion was obtained. In the other two experiments the maximum secretory rate was around 0.4 mgm. of HCl per ten minutes. Figure 1A shows one of these experiments. It can be seen that the potential of the stomach after the drop following stimulation was around 47 mv. In the other experiment the potential dropped from 70 mv. to 49 mv. and the maximum secretory rate was 0.4 mgm. of HCl per ten minutes. Atropine administration, as can be seen in figure 1A, resulted in a return of the pH to the resting level. The potential following atropine administration after a small temporary decrease returned to approximately the same level as that existing immediately before the onset of secretion.

In the above experiments with pilocarpine and mecholyl in which the maximum secretory rate was around 0.4 mgm. or less, it was found that the potential did not fall to the usual level of approximately 40 mv., but leveled off at a higher value. Comparable results have been obtained (9) with small subcutaneous doses of histamine (0.005 mgm. to 0.01 mgm. per kgm. at ten minute intervals). With histamine stimulation, however, continued administration of this substance resulted in a further lowering of the potential to the neighborhood of 40 mv. and an increase in secretory rate.

In nine of the above reported experiments in which secretion was produced by administration of pilocarpine or mecholyl, atropine sulphate was given subcutaneously or intravenously. In two of the experiments the atropine was given near the end of the secretory response, and in the other experiments it was given near the time of the maximum secretory response. The atropine sulphate was usually given intravenously in doses of 0.1 mgm. or greater. In some experiments it was given in 1 mgm. doses subcutaneously. In all of these experiments the administration of atropine was followed by a return of the potential to approximately its resting level (or higher) and by a reduction of the secretory rate to zero (pH data).

In two of the experiments the level of the potential after atropine was definitely higher than before the administration of the secretory stimulant. Interestingly in this connection it can be seen in the case of the experiment represented in figure 1C that following secretory stimulation the potential decreased and then increased to a level around 40 mv. The magnitude of this increase is essentially equal to the difference between the post-atropine level and the pre-mecholyl level. In the other experiment in which the level following atropine was higher than the original resting level, pilocarpine was used as the stimulant and the potential decreased following stimulation to approximately 40 mv. and remained there until atropine was administered. In this experiment the resting level was around 60 mv. and the post-atropine level around 75 mv.

In three experiments additional atropine was given after the potential increased and reached a relatively constant level. The additional administration of atropine was not followed by a change in potential, even when as much as 5 mgm. was given intravenously.

In the above experiments there were occasional periods after mecholyl or pilocarpine when the potential showed much more variation than with histamine. These periods usually occurred during secretion. However, in the experiment represented in figure 1A, a period of this sort occurred before the onset of secretion. The potential would show cyclic variations at the rate of about three per minute. The potential usually varied by about 2 to 3 mv. and occasionally by as much as 4 mv. These variations seem to be correlated with the variations in the mechanical activity of the stomach in the chamber, although no objective measurement of this cyclic mechanical activity was attempted.

Attempts to produce secretion in amygalized dogs by stimulation of the vagi in the neck (see Vineberg (15)), subcutaneous injection of acetylcholine, and intravenous ethyl alcohol were not successful. However, only a few experiments were performed and it is not possible on the basis of these few experiments to conclude that these procedures could not in some amygalized dogs produce secretion. In the experiments in which the vagi were stimulated the chamber technique was not used for obvious reasons.

*Effect of alcohol applied to the mucosa of the resting stomach on gastric secretion and potential.* In these experiments and those in the following section, ethyl alcohol-saline solutions were used. The chamber was drained as usual at ten-minute intervals and a given alcohol-saline solution was immediately run through

the chamber until the volume of the fluid, including that part drained from the chamber, measured 100 ml. In this way a fresh alcohol-saline solution was placed in the chamber every ten minutes. When it was desired to replace the alcohol-saline solution with 0.9 per cent saline the above procedure was repeated, only in this case saline was used in place of the alcohol-saline mixture.

The ethyl alcohol-saline solutions were made up so that there was 0.9 gram of NaCl per 100 ml. of solution, and the percentage of ethyl alcohol refers to the percentage by volume. The glass electrode was calibrated with appropriate mixtures of the ethyl alcohol-saline solutions and 0.16 N HCl.

Four dogs were used and in none of these experiments was there any evidence that the alcohol-saline mixtures resulted in the secretion of HCl. The concentration of ethyl alcohol ranged from 3.8 per cent to 47.5 per cent and was applied for periods up to 120 minutes. These solutions did, however, lower the potential. The amount the potential was lowered was dependent on the concentration of the alcohol. These points are illustrated in the experiment represented in figure 2A. It can be seen that the potential was lowered to the level of the secreting stomach, without resulting in the secretion of HCl. As can be seen in figure 2A, the stomach was still capable of secreting since histamine stimulation resulted in the secretion of HCl. There were occasional periods with alcohol-saline solutions when the titratable acidity increased above that of the resting level (see fig. 2A). The pH, however, showed no tendency to decrease and it is assumed that the increase in the titratable acidity was due to the liberation of substances other than HCl.

Obviously, it cannot be concluded from these experiments that the application of alcohol to the mucosa would not evoke secretion in amyotomized dogs under these conditions, if applied for longer periods of time or if the alcohol solutions did not contain 0.9 of a gram of NaCl per 100 ml.

*Effect of ethyl alcohol on the potential and secretory rate of the secreting stomach.* In previously reported experiments it was found that the application of relatively concentrated HCl solutions (9) or the passage of a direct electric current from the mucosa to the serosa (10) resulted in a lowering of both the potential and secretory rate. In these experiments the secretory rate was not measured during the exposure of the mucosa to the HCl solutions, but only after replacement with saline, and in the current sending experiments the potential was not measured during the passage of the current. It would be of interest to determine the correlation between secretory rate and potential with another agent that lowered the potential, particularly one that did not interfere with the measurement of either the secretory rate or the potential. Ethyl alcohol was found to be such an agent, and its effects on the secretory rate and potential are presented in this section. A total of seven dogs was used in these experiments, and histamine was used as the gastric stimulant.

Figure 3A represents an experiment in which 17.1 per cent alcohol-saline was applied for 30 minutes after the secretory rate had reached a relatively constant level. It can be seen that both the secretory rate and potential decreased while the alcohol was in contact with the mucosa, and that after replacement with saline the potential and secretory rate both gradually increased.

The secretory rate returned to approximately its pre-alcohol level while the potential reached a value lower than its pre-alcohol level.

Figure 3C represents an experiment in which the concentration of alcohol was gradually increased. It can be seen that as the potential decreased the secretory rate also decreased. Within the limits of the normal variation of the potential and secretory rate, it can be seen that the first definite decrease in potential is attended by a decrease in the secretory rate. It can also be seen that as the potential continued to decrease, the secretory rate also continued to decrease. Replacement of the alcohol solutions with saline is again followed

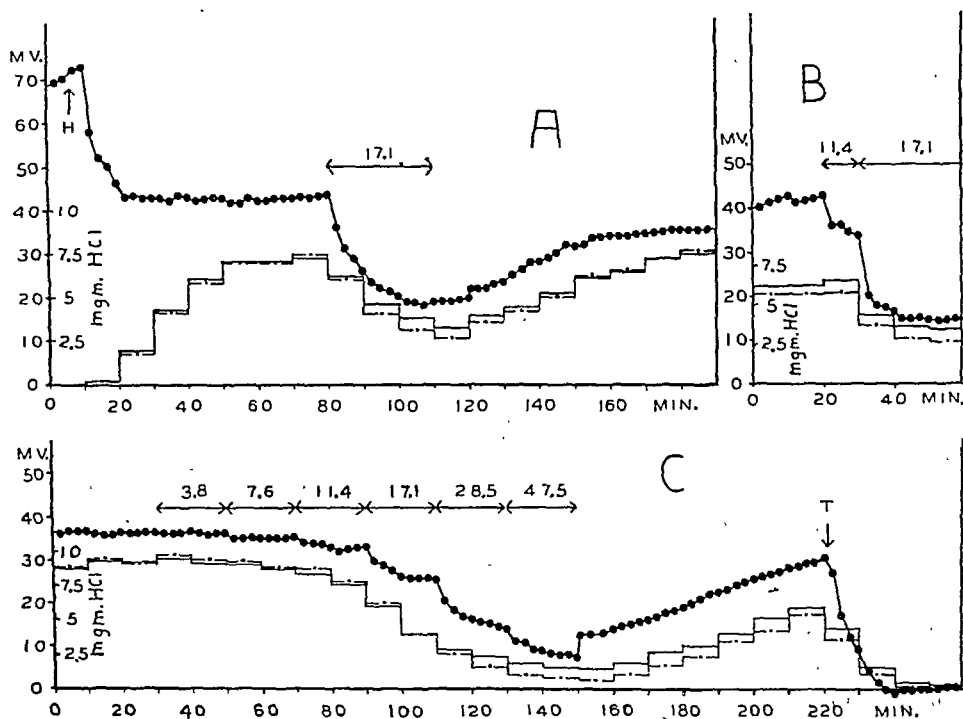


Fig. 3A, B, and C. The same conventions for secretory rate used as in figure 1C. Same conventions for alcohol-saline solutions as in figure 2. In all of the experiments represented in this figure,  $\frac{1}{2}$  mgm. of histamine diphosphate was injected subcutaneously every ten minutes, except that the first injection of histamine in figure 3A at time indicated by arrow H was 1 mgm. At time indicated by arrow labeled T in figure 3C the trachea was clamped.

by a concomitant increase in both the secretory rate and the potential. The dog's trachea was clamped at the time indicated in the figure, and the heart stopped beating a few minutes later. The secretory rate and potential both decreased to zero.

Figure 2B shows an experiment in which the application of 3.8 per cent alcohol was followed by a small, but definite, decrease in the potential while the secretory rate continued to increase. However, a further lowering of the potential following the use of 7.6 per cent alcohol-saline resulted in a decrease in the secretory rate.

In figure 2A, it will be recalled, the potential was depressed by the applica-

tion of alcohol to the resting stomach. Injection of histamine during this depressed period resulted in the secretion of HCl, and as the secretory rate increased the potential also increased. It can also be seen in this figure that the application of 17.1 per cent alcohol-saline to the mucosa, before the secretory rate leveled off, resulted in a decrease of the potential of approximately 15 mv. and a definite decrease in the secretory rate.

The above experiments demonstrate that in general in the secreting stomach, after the secretory rate has reached a relatively constant level, a depression of the potential following alcohol application to the mucosa is associated with a decrease in the secretory rate. The most outstanding exception to this rule in all of the experiments in which alcohol was used is shown in figure 3B. In this experiment the use of an 11.4 per cent alcohol-saline solution resulted in a decrease in the potential of approximately 6 mv., while the secretory rate did not decrease. In fact, the titratable acidity showed a slight increase. However, subsequent application of a 17.1 per cent alcohol-saline solution resulted in a decrease of both the secretory rate and the potential.

*The rôle of the liquid junction potential (diffusion potential) between the gastric juice and the fluid in the chamber in the decrease of the potential of the secreting stomach following the use of alcohol-saline solutions.* Previously published experiments (11) have demonstrated that in the resting stomach the electromotive force (or forces), giving rise to the potential difference across the stomach, arises somewhere in the mucosa (including of course the interface between the mucosa and external fluid). In the secreting stomach, as was pointed out previously (9), there is undoubtedly a diffusion potential at the junction of the gastric secretion and the chamber fluid, in addition to the electromotive force (or forces) arising in the mucosa. The gastric secretion diffuses outward into the fluid of the chamber and hence this junction would be formed at a distance from the actual surface of the mucosa. Therefore, this junction would be in an aqueous medium and the orientation of its potential would be such as to tend to make the mucosal electrode positive to the serosal electrode in the external circuit. Since the overall potential has the opposite orientation (the serosal electrode is positive in the external circuit to the mucosal electrode), it follows that an increase in the magnitude of this diffusion potential, by itself, would result in a decrease in the overall potential and vice versa.

The question arises as to whether the decrease in the potential of the secretory stomach, as a result of placing alcohol-saline solutions in the chamber, is due to an increase in this diffusion potential. In an attempt to answer this question, the diffusion potentials between 0.16 N HCl and alcohol-saline solutions and between 0.16 N HCl and 0.9 per cent saline were measured. The HCl solution was placed in a J-shaped glass tube with a diameter of approximately 4 mm., and this tube was carefully lowered into a much larger vessel containing either saline or an alcohol-saline solution. Saturated KCl solutions were used to connect these two solutions to non-polarizable electrodes. The assumption is made that the potentials at the saturated KCl junctions are zero (but see MacInnes and Longworth (5)). With this method the potentials reached a relatively

constant level within a matter of minutes. For the sake of comparison the value measured five minutes after the junction was formed was taken as the magnitude of the diffusion potential. The procedure was repeated at least three times for each solution. The three values for each alcohol-saline solution are given in table 1, together with their averages. The difference between the diffusion potential of 0.16 N HCl and saline and the diffusion potentials of the various alcohol-saline solutions is also given in the table. These experiments were carried out at room temperature (approximately 22°C.). Although the temperature of the fluids in the chamber was approximately 39°C., the difference between the diffusion potentials at the two temperatures on the basis of theoretical considerations would be unimportant from the point of view of these experiments. This was confirmed by a few experiments on the diffusion potentials at the higher temperature.

TABLE 1

*Diffusion potentials between 0.16 N HCl and 0.9 per cent saline or alcohol-saline solutions*

The first row gives the composition of the solution. The percentages represent the amount of alcohol on a volume basis. All of the solutions contained 0.9 gram of NaCl per 100 ml. The second, third, and fourth rows give the values for the diffusion potentials in mv. for three separate determinations, and the next row the averages. The last row gives the difference between the diffusion potential between 0.16 N HCl and a given alcohol-saline solution and the diffusion potential between 0.16 N HCl and 0.9 per cent saline.

	SALINE	3.8%	7.6%	11.4%	17.1%	28.5%	47.5%
	30.2	31.4	31.8	33.1	34.0	36.8	43.4
	30.1	31.2	31.8	33.1	34.6	37.1	43.2
	30.4	31.0	32.0	33.0	34.0	37.7	42.9
Ave.....	30.3	31.2	31.9	33.1	34.2	37.2	43.2
Diff.....		0.9	1.6	2.8	3.9	6.9	12.9

It can be seen that in those experiments (figs. 2 and 3) in which there was a marked drop in the potential and the secretory rate, the marked decrease in the potential was greater than the increase in the diffusion potential that might be expected from the values given in table 1.

It is well known that in measurements of diffusion potentials the magnitude of a given diffusion potential is a function of the type of junction produced. However, the variations in the magnitude of diffusion potentials from one method to another are usually only a matter of a few millivolts (see 14).

Another important factor is obviously the concentration of HCl in the gastric juice. It is assumed that the maximum possible concentration of HCl in gastric juice is in the neighborhood of 0.16 N (see Hollander (4), and Gray (2)). However, in most of the experiments reported in the literature the actual concentrations are usually found to be less than 0.16 N HCl. Therefore, as far as this factor is concerned, the values in table 1 can be regarded as the maximum or near the

maximum for the magnitude of the diffusion potentials between the gastric secretion and the fluid in the chamber.

There is a real possibility that there may be islands in the gastric mucosa where HCl is not formed. Therefore, if there were not a more or less continuous interface between the HCl of the gastric secretion and the chamber fluid the resultant effect on the overall potential, on the basis of the laws of electrical networks, would be less than the magnitude of the diffusion potential measured between gastric juice and chamber fluid, with the technique described above.

On the basis of the foregoing analysis, the conclusion would be warranted that the difference between the diffusion potential between 0.16 N HCl and saline and between 0.16 N HCl and a given alcohol-saline solution is as great (probably greater) than the change in the diffusion potential in the chamber resulting from the use of a given alcohol-saline solution.

Therefore, in these experiments in which there was a relatively marked decrease in the potential it is possible to conclude that only a part of the decrease is due to a change in the diffusion potential. For instance, in figure 3A, application of 17.1 per cent alcohol-saline resulted in a decrease of approximately 24 mv., while the maximum expected change in the diffusion potential (table 1) is in the neighborhood of 4 mv. In the experiment represented in figure 3C the potential gradually decreased as the concentration of alcohol was increased and was approximately 30 mv. less than its original value when 47.5 per cent alcohol-saline was in the chamber. The maximum expected change in diffusion potential with 47.5 per cent alcohol-saline would be (table 1) approximately 13 mv. It can be seen that there was a sharp rise of approximately 5 mv. in this experiment after saline replaced the alcohol solution. Although it is not conclusive, this sharp rise of 5 mv. suggests that the actual effect on the overall potential of the change in diffusion potential in this case is actually 5 mv., which is a little less than half the maximum expected value from table 1.

If the decrease in the potential following the use of alcohol-saline solutions was due solely to a change in the magnitude of the diffusion potential, then replacement of the alcohol-saline solutions with 0.9 per cent saline ought to restore immediately the overall potential to its original value. This never occurred. In fact, the greatest immediate increase after replacement with 0.9 per cent saline was found in the experiment represented by figure 3C. The fact that in every experiment the potential, following replacement with 0.9 per cent saline, only slowly returned to its level before application of alcohol-saline solutions is additional evidence that the depression of the potential is not primarily due to an increase in the diffusion potential.

In some of the experiments (see fig. 3C) the changes in potential following alcohol-saline solutions were of approximately the same order of magnitude as the values of the diffusion potentials given in table 1. However, it would appear from these data that the major portion of the change in the potential resulting from the use of alcohol-saline solutions, when there is a relatively marked decrease in the potential, is due to the effect of alcohol on the mucosa and not to an increase in the diffusion potential.

DISCUSSION. The above experiments demonstrate that the relationship between the gastric potential and the rate of secretion of HCl after pilocarpine or mecholyl stimulation is essentially the same as that found after histamine stimulation. They demonstrate that a decrease in the potential is associated with the onset of secretion, and that after the initiation of secretion the secretory rate may undergo wide variations while the potential shows relatively little change. They also show that stimulation resulting in very low secretory rates is associated with a smaller drop in potential than that which occurs with higher secretory rates.

It was found that the administration of atropine to pilocarpine or mecholyl stimulated stomachs results in a reduction of the secretory rate to zero and an associated rise in the potential. These findings are quite similar to those previously reported (12) in which the administration of thiocyanate to histamine stimulated stomachs also resulted in a decrease of the secretory rate to zero and an increase of the potential. It should be pointed out in this connection that Crane, Davies and Longmuir (1) have reported in recent work on the gastric mucosa of the frog a relationship between the secretion of HCl and the gastric potential similar to that found by the present writers. They found that the onset of gastric secretion is associated with a drop in potential and that thiocyanate inhibits secretion and produces a concomitant rise in the potential. In fact most of the investigators working in this field have reported a change in the potential with the onset of secretion (see (9) for a detailed discussion of the literature). However, Quigley, Barcroft, Adair and Goodman (8) have reported that injection of atropine, pilocarpine, or histamine in unanesthetized pouch dogs did not result in a change in the potential. It is difficult to evaluate the work of this group since they did not measure the secretory rate. They also used a technique for measuring the potential that has certain inherent errors in it (see (13) for a detailed discussion of this technique). In spite of these errors, it is probable that certain of their results are essentially valid. For instance, on the basis of the work of the present writers, administration of pilocarpine or histamine would not be expected to result in a significant change in the potential unless the secretory rate was initially very low or zero, and the fluid in contact with the mucosa was less acid than normal gastric juice. Similarly, the administration of atropine would not be expected to result in a significant change in the potential unless the administration of atropine resulted in a reduction of the secretory rate to practically zero and the fluid in contact with the mucosa was replaced with a fluid less acid than gastric juice. Obviously further work should be done on the unanesthetized dog's stomach in which both the secretory rate and the potential are measured, the potential being measured with a method (see (13)) that eliminates the errors of the technique employed by Quigley et al. (8).

The experiments in the present paper also demonstrate that the application of alcohol to the resting stomach resulted in a lowering of the potential to levels that occur during secretion, without producing secretion. Mislowitzer and Silver (6) found that ethyl alcohol applied to the mucosa of the cat's stomach

resulted in a decrease of the potential. These investigators, however, did not measure the secretory rate.

Previous experiments have demonstrated that the lowering of the potential of the resting stomach to secretory potential levels, following the application of relatively concentrated HCl solutions and subsequent replacement with saline (9) or by application of a direct electric current (10), did not result in secretion. In many observations on the potential of the non-secreting stomach, following interruption of the blood supply to the stomach, it was found that in every case the potential decreased to approximately zero over the course of about 30 minutes or less. This decrease in potential was never associated with secretion. On the basis of the above, the conclusion is warranted that a number of procedures may reduce the potential of the resting stomach to secretory levels without producing secretion.

It was also found that histamine stimulation, following a marked depression of the potential of the resting stomach by the application of alcohol, resulted in secretion and, as the secretory rate increased, the potential also increased and approached the level of the potential of the secreting stomach in which there had been no previous depression of the resting potential. Essentially this same relationship has been found after histamine stimulation in stomachs in which the potential had been depressed by application of current from mucosa to serosa (10), or by the application of HCl and subsequent replacement with saline (9).

It was also found that after the secretory rate has reached a relatively constant level a reduction of the potential, as a result of the application of alcohol to the mucosa, results in a lowering of the secretory rate. There were exceptions to this (fig. 2B and fig. 3B), but in these cases further lowering of the potential was associated with a decrease in the secretory rate. It was also established that the decrease in the potential (certainly when the decrease was marked), after alcohol-saline solutions, was primarily due to the effect of alcohol on the mucosa and not to an increase in the magnitude of the diffusion potential between the gastric juice and chamber fluid. In previously reported experiments it was found that the lowering of the potential of the secreting stomach, either by application of HCl solutions and subsequent replacement with saline (9) or by sending electric current from the mucosa to serosa (10), also resulted in a decrease of the secretory rate. Therefore, it has been established with several different procedures that lower the potential, that there is a relatively good correlation between the potential of the secreting stomach and the secretory rate. Interestingly in this connection, Crane, Davies and Longmuir (1) found that KCN or iodoacetate depresses both the potential and secretory rate of the frog's gastric mucosa.

In our opinion, the results on the relationship between the secretory rate and potential, summarized above, give under the experimental conditions a fairly comprehensive picture of this relationship. The fact that there is a fairly good correlation between a reduction in the secretory rate of the secreting stomach and a decrease in the magnitude of the potential suggests that the potential

might play a rôle in the secretion of HCl. However, the observation that the potential may show relatively little change while the secretory rate varies over a relatively wide range might be interpreted, on superficial analysis, to mean that the increase in the output of HCl was not attended by an increase in electrical energy expenditure. However, (see (12) for a more detailed discussion of this point) it must be kept in mind that the potential difference across the stomach wall is not a measure of the electrical energy output. In unpublished work it has been shown that the mucosa of the stomach can produce under certain conditions approximately as much electrical energy, on a per gram basis, as the electric organ of *Electrophorus electricus* (data of Nachmonsohn, Coates, Rothenberg, and Brown (7)).

#### SUMMARY

1. Pilocarpine or mecholyl administration in dogs under certain experimental conditions results in a decrease of the potential difference across the stomach wall. This decrease is associated with the onset of secretion of HCl. After the initial decrease of the potential the potential may show relatively little change while the secretory rate may increase considerably.

2. Administration of atropine, after secretion has been initiated by pilocarpine or mecholyl, results in a decrease in the secretory rate to zero and a concomitant increase in the potential.

3. Ethyl alcohol-saline solutions, applied to the mucosa of the stomach for certain periods of time, did not under the conditions of the experiments result in the secretion of HCl. The potential on the other hand was lowered by this procedure.

4. A good correlation was found between the secretory rate and potential following application of alcohol-saline solutions to the mucosa in histamine stimulated stomachs. A marked lowering of the potential was always associated with a marked lowering of the secretory rate. Experiments were performed in which the diffusion potentials between 0.16 N HCl and 0.9 per cent saline and between 0.16 N HCl and alcohol-saline solutions were measured. The difference between these diffusion potentials was, in general, definitely less than the decrease in the gastric potential. It was concluded that the decrease in the gastric potential was primarily due, in those cases where the decrease was relatively marked, to the effect of the alcohol on the gastric mucosa and not to an increase in the diffusion potential between the gastric solution and chamber fluid.

5. The results of the present investigation are discussed in the light of previously published work. It is concluded that the results of the present work, taken together with those previously published, give under the conditions of the experiments a fairly comprehensive picture of the relationship between the rate of secretion of HCl and the potential.

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# THE EFFECT OF MUSCULAR FATIGUE ON THE SOLUBILITY OF MYOSIN

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The relationship of muscular fatigue to the solubility of myosin was first studied by Deuticke (1) who reported decreased solubility in proportion to the degree of fatigue. A number of reports has confirmed this observation (2, 3, 4, 5, 6). Others have failed to confirm it (7, 8). Decreased solubility has been ascribed to coagulation, but it has been shown (4) that it is not identical with denaturation coagulation because there is no demonstrable increase in S—S and SH groups in myosin in rigor, such as occurs in denaturation. Dehydration coagulation most nearly reproduces all the phenomena of fatigue coagulation.

It is the purpose of this paper to present the results of some experiments done in an effort to re-examine this problem by some variations in technical procedures, namely, freezing in fatigue and tetanic states.

Both frogs and rats were used, since myosin is not species-limited. In case of the former, the gastrocnemius muscle was dissected free, the circulation interrupted, and the muscle stimulated with 1-second induction shocks while loaded optimally. Upon failure to respond to maximal shocks, the muscle was removed quickly, frozen in a dry ice-alcohol mixture, weighed, ground in a chilled mortar and washed into a 250 cc. Erlenmeyer flask, an extraction solution added cold and the flask shaken in an ice bath for 60 minutes. The resulting suspension was filtered through a Buchner funnel under vacuum and the paper washed. The filtrate was analysed by Kjeldahl. The extraction, 0.5 M KCl and 0.03 M NaHCO<sub>3</sub>, was described by Bailey (9).

In a test of accuracy, both gastrocnemii were removed from 10 frogs after anesthesia and processed separately. The mean nitrogen yield was 1.01 grams per cent from the right and 1.00 gram per cent from the left. The range among all samples was 0.72–1.32 grams per cent. The maximum difference between muscles from the same animal was 0.16 gram per cent. The table of data is omitted to conserve space, but statistical treatment shows Fisher's T value to be 0.089; therefore, differences as determined are random and insignificant.

In the first experimental group of 9 frogs, one gastrocnemius was removed, stimulated to fatigue (5–10 min.) in a moist chamber, the other processed immediately as a control. However, instead of a 60 minute period of mechanical agitation, a 24 hour period of occasional gentle stirring of the brei was used. Because of the extremely erratic and inconsistent results, the first procedure described was used in all subsequent experiments.

In the second group of 13 frogs, the mean yield of nitrogen from the control

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muscles was 1.11 grams per cent and 1.03 grams per cent in the exhausted group, a decrease of only 0.08 gram per cent which gives  $T = 0.73$ , which is not a statistically significant difference.

Deuticke had indicated that the degree of decrease in protein solubility is directly proportional to the amount of work done before fatigue, that is, to the endurance of the muscle. To test this point, the third experimental group of 9 frogs was treated as follows: The muscle to be fatigued was left in situ, protected by the skin, but the circulation was occluded and the load attached to the exposed tendon. It was felt that the muscle might survive for a longer time and thus accumulate more work. This assumption proved correct, as all muscles survived nearly twice as long. The mean nitrogen extraction from the control muscles was 1.20 grams per cent to 0.98 gram per cent in those fatigued, a difference of 0.22 gram per cent. The data gave  $T = 2.48$  which is strongly significant, statistically. This last group, when compared to the controls, confirms Deuticke's findings.

Rats were now used. In the first group of 7 animals, one gastrocnemius was dissected free but left in situ and protected by the skin. A 50 gram weight was attached to the tendon. It required from 60 to 90 minutes to bring on fatigue with single induced shock of approximately 1 per second. The mean nitrogen yield from the control muscles was 1.69 grams per cent and 1.48 in the fatigued group, a difference of 0.21 gram per cent;  $T = 1.099$  which is statistically insignificant.

In a second group of 12 rats, one muscle was fatigued by single shocks at 1 second intervals, 60 to 90 minutes. Upon complete failure to respond to this stimulus, it was tetanized for 60 seconds; then while still subject to this stimulus, was dipped into the freezing mixture. The nitrogen yield from the controls was 1.36 grams per cent and 1.18 from the experimentals, a difference of 0.18 gram per cent;  $T = 3.85$ , statistically a highly significant difference.

In a third group of 8 rats, the experimental muscle was tetanized immediately and while in that state frozen before any degree of fatigue could occur. The average for the controls was 1.39 grams per cent N, and for the experimentals 1.50 grams per cent,  $T = 0.769$ , which is not significant.

Since the possibility of the influence of vitamin deficiency on muscular function has not yet been disposed of, it seemed worthwhile to test this point. Eight litter mates of the first group of rats were kept on a vitamin B-free diet for 1 month. The stimulated muscles reacted as long as those of the control group. The mean extraction of N was 1.84 grams per cent in the controls and 1.51 grams per cent in the experimentals.  $T = 2.60$ , which is statistically significant.

The effect described by Deuticke was confirmed in only 1 group of frogs, although the small differences observed in the other are uniformly in the same direction. In the first experimental group, the data were discarded for purposes of this discussion.

The Deuticke effect also was found in only one rat group, that in which the muscle was frozen while in a tetanic state following fatigue due to repetitive stimulation. It is obvious that the duration of exercise preceding fatigue is an

important factor. A fatigue period embracing 300-600 contractions produced much less decrease in solubility of myosin than one of 4000 contractions. Also, the difference is demonstrable only after alkaline extraction, possibly because acid solutions penetrate the sarcolemma of the fibrils and in that way increase the yield of nitrogen. Therefore, the exact method of extraction becomes a limiting factor of major importance. This has been interpreted also as indicating the formation of alkaline myosin in fatigue.

It has been suggested by Needham (9) that myosin in a supercontracted state might accept phosphate from adenosine triphosphate, then extend, releasing the phosphate upon subsequent contraction. In fatigue then, myosin might be unable to accept phosphate from adenosine triphosphate and so would remain in a super contracted state.

The state of contraction of myosin per se does not seem to be a factor in extractability, for there was no decrease in solubility in the last rat group, from which the muscle was frozen while tetanized without fatigue.

While the actual mean recovery of nitrogen from both sets of muscles of the deficient animals was higher than for healthy animals (group 2), the difference was of less significance. The higher yield was due to the relative increase in protein at the expense of other constituents, such as glycogen.

The results of these experiments offer no explanation of this phenomenon.

#### SUMMARY

1. In both frogs and rats, alkaline extraction of muscles fatigued by induction shocks yielded less nitrogen than rested muscle, confirming the Deuticke effect.
2. The decreased extractability of nitrogen was generally proportional to the period required for fatigue.
3. Rested muscles frozen while in tetanus showed no decrease in extractable nitrogen.
4. Vitamin B deficiency did not affect the extractability of nitrogen, although there was a greater yield from both sets of muscles.

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# THE EFFECT OF HEAVY MUSCULAR WORK ON THE VOLUME OF CIRCULATING RED CORPUSCLES IN MAN

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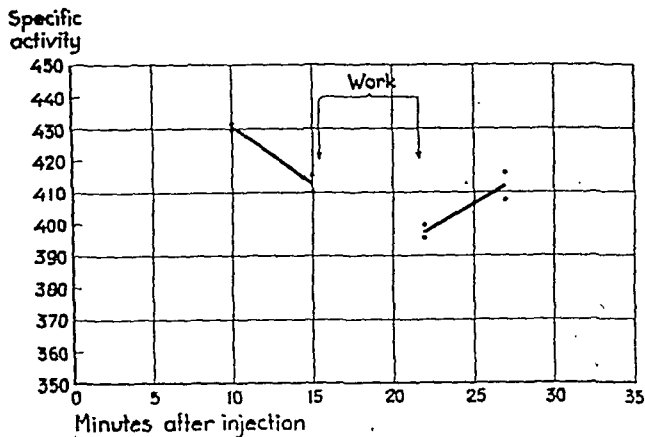
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The classical investigations carried out by Barcroft and his associates (1, 2, 3) into the function of the spleen as a blood reservoir in dogs aroused the interest of other workers in similar experiments on human beings. Thus Arnold and Krzywaneck (4) observed a considerable increase in the number of red corpuscles after muscular effort. Barcroft's findings have been confirmed by a number of workers, notably Binet and his associates (5, 6). Other organs have also been supposed to act as reservoirs. Thus Wollheim (7) suggests the sub-papillary plexus, Krogh (8) the portal system and Sjöstrand (9) the lungs.

There have been very few investigations into the volume of blood in circulation before, during and after exercise in man. Asmussen (10) using the CO-method has, it is true, found no increase in the volume of blood in circulation during light muscular work (360 mkg.) but this method is a questionable one, because as Asmussen himself admits, "an unpredictable amount of CO is taken up by myoglobin."

**RESULTS.** As Nylin (11,12,13) has already shown, the method of labelling corpuscles with radioactive phosphorus is very suitable for studying fluctuations in the circulatory corpuscular volume during at least one hour after intravenous injection of the labelled corpuscles. In an earlier paper (14) the author has shown that in six normal persons the specific activity of the labelled corpuscles remained remarkably constant from the 5th to the 60th minute after injection. This made it possible to study changes in the circulatory corpuscular volume, especially after muscular work, within at least one hour after the injection of labelled corpuscles.

Five policemen, 30-40 years of age and perfectly healthy, were chosen for the experiment. Early in the morning, the subject was placed in the recumbent position and the labelled corpuscles were injected into the median cubital vein. Double blood samples were taken from the vein of the opposite arm 10 and 15 minutes respectively after the injection. At the end of the 15th minute the subject had to do severe muscular work on the stairs (15); and 20 rounds at a rate of 208 steps per minute. This work is very strenuous and can hardly be performed by anyone who is not in good training. At the 25th and 32nd minute after the injection double samples were taken from the median cubital vein. A second double sample was taken at the 27th to the 39th minute. The activity of all the samples was then measured in the usual manner with the Geiger counter and the specific activity calculated. The results before and after work in the case of each of the five normal persons are shown in figures 1-5,



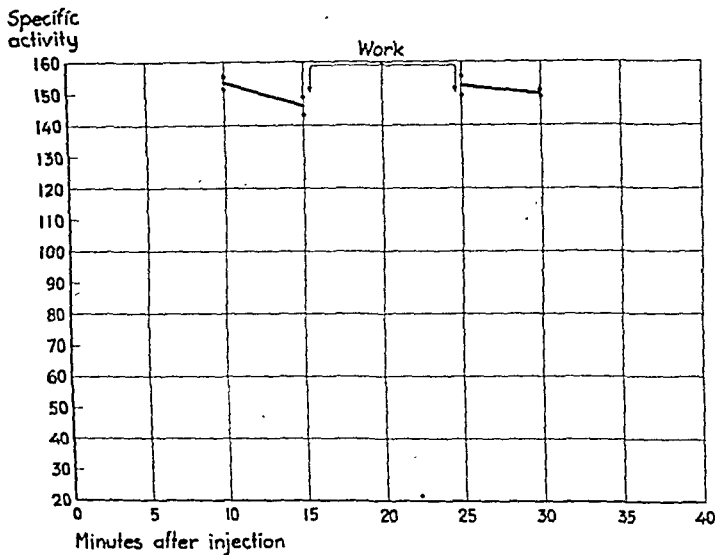
Before work (Hematocrit 43 %)

Red blood corpuscles	1750 gr	33 gr/kg	1620 ml	30 ml/kg
Plasma	2325	44	2280	43
Circulat. bl. volume	4075	77	3900	73

After work (Hematocrit 45 %)

Red blood corpuscles	1825 gr	34 gr/kg	1700 ml	32 ml/kg
Plasma	2230	42	2185	42
Circulat. bl. volume	4055	76.5	3885	74

Case 1. Blood volume before and after hard work in a normal person.



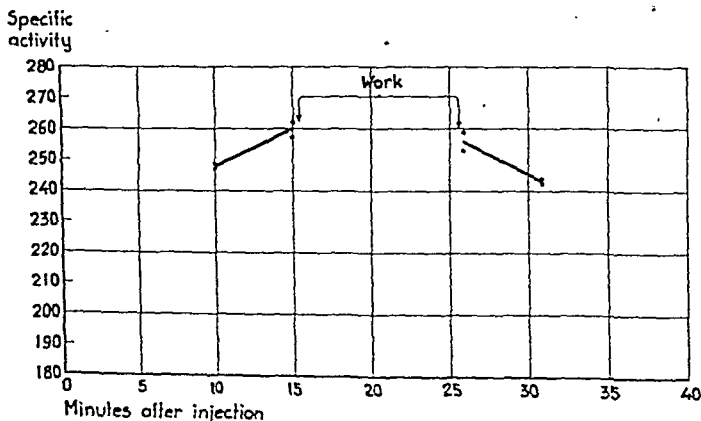
Before work (Hematocrit 48.5 %)

Red blood corpuscles	3240 gr	38 gr/kg	3000 ml	35 ml/kg
Plasma	3440	40.5	3380	40
Circulat. bl. volume	6680	78.5	6380	75

After work (Hematocrit 49 %)

Red blood corpuscles	3200 gr	38 gr/kg	2960 ml	35 ml/kg
Plasma	3320	39	3260	38
Circulat. bl. volume	6520	77	6220	73

Case 2. Blood volume before and after hard work in a normal person.



Before work (Hematocrit 48 %)

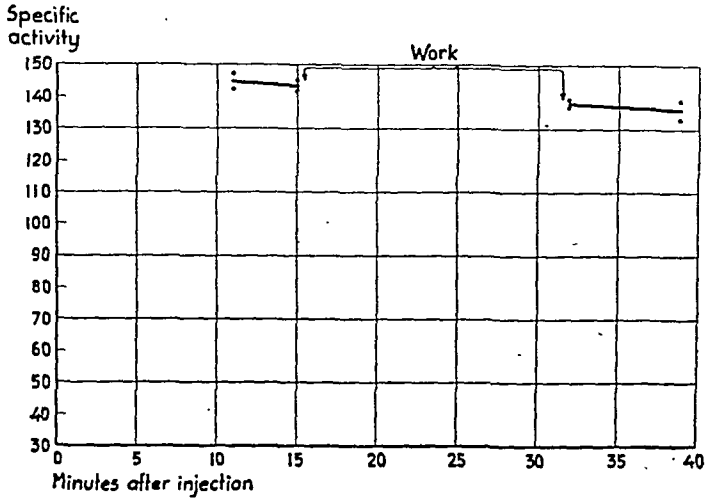
Red blood corpuscles	2170 gr	26 gr/kg	2010 ml	24 ml/kg
Plasma	2350	28	2300	28
Circulat. bl. volume	4520	54	4310	52

After work (Hematocrit 50 %)

Red blood corpuscles	2210 gr	26 gr/kg	2050 ml	25 ml/kg
Plasma	2210	26.5	2160	26
Circulat. bl. volume	4420	53	4210	51

Case 3. Blood volume before and after hard work in a normal person.

and tabulated in table 1. There are very small variations in the specific activity of the double samples. The error of the method has been statistically calculated and it amounts to 3.7 impulses, i.e., 1.2 per cent of the general mean, which is very little. The difference in the specific activity of the general mean before and after work amounts to 5 impulses/minute, which must be regarded as a



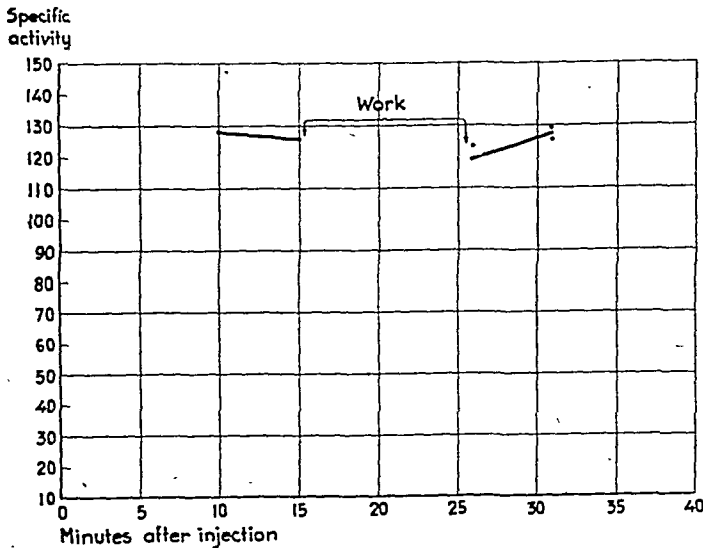
Before work (Hematocrit 46 %)

Red blood corpuscles	2510 gr	29 gr/hg	2320 ml	27 ml/hg
Plasma	2950 • 34 •	2890 • 33 •		
Circulat. bl. volume	5460 • 63 •	5210 • 60 •		

After work (Hematocrit 48 %)

Red blood corpuscles	2660 gr	30.5 gr/hg	2460 ml	28 ml/hg
Plasma	2800 • 33 •	2820 • 32 •		
Circulat. bl. volume	5540 • 63.5 •	5280 • 61 •		

Case 4. Blood volume before and after hard work in a normal person.



Before work (Hematocrit 46.5 %)

Red blood corpuscles	2370 gr	30.5 gr/hg	2200 ml	28 ml/hg
Plasma	2730 • 55 •	2670 • 34.5 •		
Circulat. bl. volume	5100 • 65.5 •	4870 • 62.5 •		

After work (Hematocrit 50 %)

Red blood corpuscles	2460 gr	31.5 gr/hg	2270 ml	29 ml/hg
Plasma	2460 • 31.5 •	2410 • 31 •		
Circulat. bl. volume	4920 • 63 •	4680 • 60 •		

Case 5. Blood volume before and after hard work in a normal person.

very small difference and not statistically significant. As has been shown before we could not find any decrease in activity in 6 other normal persons up to one hour after injection. Unfortunately, in the five policemen in this material the activity was not followed to the end of the hour. It appears as if the injected corpuscles had become mixed up with all the circulatory blood corpuscles

of the body by the 10th-15th minute, and that after hard work no further dilution was caused by the emptying of a blood depot (table 1). Table 2 shows the calculated weight of the red corpuscles and the calculated total blood volume before and after work. The mean weight of the red corpuscles before work is 2408 grams and after work 2471 grams, the difference being 63 grams. This dif-

TABLE 1

*Specific activity of red blood corpuscles in the venous blood 10-39 minutes after injection of labelled corpuscles before and after hard work in five normal persons*

CASE NUMBER	BEFORE WORK		AFTER WORK	
	10 min.	15 min.	25-32 min.	27-39 min.
1.	431	410	396	407
	430	417	404	416
2.	151	151	155	149
	155	143	149	151
3.	247	257	252	243
	248	262	258	243
4.	142	145	137	139
	148	142	139	133
5.	128	126	115	129
	128	126	123	125
Mean.....	220	218	213	214
General mean.....	219		214	

TABLE 2

*Circulating blood volume before and after hard work in five normal persons*

CASE NUMBER	WEIGHT KG.	BEFORE WORK					AFTER WORK				
		Red corpuscles		Hema- tocrit	Blood volume		Red corpuscles		Hema- tocrit	Blood volume	
		gr.	gr./kg.	%	cc.	cc./kg.	gr.	gr./kg.	%	cc.	cc./kg.
1.	53	1750	33	43	3900	74	1825	34	45	3885	74
2.	85	3240	38	49	6380	75	3200	38	49	6220	73
3.	83	2170	26	48	4310	52	2210	27	50	4210	51
4.	87	2510	29	46	5210	60	2660	31	48	5280	61
5.	78	2370	31	47	4870	63	2460	32	50	4680	60
Mean.....	77	2408	32	47	4934	65	2471	33	49	4855	64

ference may not be statistically significant according to the statistical treatment of the figures in table 1. The hematocrit reading before the work is 47 and after the work 49, a very small difference. The plasma volume has been calculated and so also the total circulatory blood volume. The mean circulatory blood volume before the exercise amounts to 4934 cc. and after the exercise to 4855 cc., which means that the total circulatory blood volume has decreased

by 79 cc., which probably is not statistically significant. The decrease probably depends in part on loss of fluid from the circulation, and this explanation is supported by the small increase in the hematocrit reading after the exercise.

#### SUMMARY

We may conclude from this investigation, in which the weight of the circulating red corpuscles has, with the help of tagged erythrocytes, been determined before and after work that there is no reservoir which empties red corpuscles into the blood stream after work, or, if there is, it is too small to be shown statistically. This uncertainty regarding the existence of a reservoir in man stands in contrast to the results obtained by Barcroft with dogs.

I wish to express my sincere thanks to the Swedish Board of Medical Research, the financial assistance of which has enabled me to carry out this investigation.

I beg to extend my warm thanks to Prof. G. de Hevesy for his unfailing interest and to Prof. Manne Siegbahn, who kindly placed radioactive phosphorus at my disposal.

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# THE EFFECT OF EXPERIMENTAL INSOMNIA ON THE RATE OF POTENTIAL CHANGES IN THE BRAIN<sup>1</sup>

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It may be said that no significant physiological or biochemical changes have been observed during periods of experimental insomnia, even up to 200 hours. Blood sugar, alkaline reserve, hemoglobin, red and white cell count, body weight, basal metabolism and body temperature show little variations from the normal (14, 11, 15, 10). The excretion of 17-ketosteroids (21), the level of adrenaline-like substances in the blood (17), and the excretion of total nitrogen and creatinine (11, 20) are little affected. Nor are there any significant changes in auditory acuity (8), static ataxia, flicker fusion frequency (20), or reflexes (11, 6, 20). Respiration, heart rate and blood pressure changes that occur are slight and usually can be attributed to the state of relaxation of the sleepy subject (11, 6, 20).

Much emphasis has been placed on the psychomotor changes but those that do occur are either of small magnitude or do not lend themselves readily to objective analysis. They consist chiefly in a somewhat slower reaction time, and particularly in the subject's inability to maintain sustained effort and performance (14, 11, 10, 6, 20).

The most marked changes that occur are the psychological disturbances. These alterations in behavior become manifest usually between 36 to 60 hours of sleeplessness, and are characterized by an increased irritability, loss of memory, a tendency towards hallucinations and illusions, inattention, general indifference (14, 11, 6, 10) and, in some instances, symptoms resembling acute schizophrenia are produced (19, 20).

In order to obtain more objective methods to study the effects of the fatigue of sleeplessness new procedures are necessary. Some investigations (2, 3) indicate that definite qualitative changes in the EEG occur as a result of voluntary insomnia. Since it appears that the effects produced by prolonged wakefulness are principally confined to the brain a quantitative study of the brain potential patterns in such situations seems indicated.

This report deals with the percentage time occupied by each frequency from 2 to 24 per second at 50, 75 and 100 hours of experimental insomnia.

**METHODS.** *General methods.* Twelve subjects, ranging from 18 to 33 years of age, were used. All remained awake for 112 hours. The methods of keeping them awake and their activities during that period are described elsewhere (20, 21).

<sup>1</sup> The work described in this report was done under contract recommended by the CMR, between the Office of Scientific Research and Development and the California Institute of Technology.

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Control recordings were taken 3 days prior to the experimental period. The subjects were then divided into three groups of 4 men each and records were again taken on one group at 50 hours, a second group at 75 hours and a third group at 100 hours of insomnia. Although the electrical activity over the right and left frontal, parietal, temporal and occipital cortex, as well as the vertex was recorded, using both monopolar (indifferent electrodes on the ears) and bipolar leads, only records obtained from the right and left fronto-occipital leads were quantitatively analyzed. All EEGs were taken with the subject seated, eyes closed—but awake. It is important to emphasize this last point. The potential changes thus recorded at the various intervals of sleeplessness are to be considered "waking" potentials at those times. This was accomplished through the excellent co-operation of the subjects.<sup>3</sup> Occasionally, a subject would start to doze off and had to be awakened, but only a minimum of prodding nudges, etc., had to be given. When this occurred, that portion of the record was marked off and subsequently not used in the analysis. Upon dozing, the record showed the slow activity waves characteristic of sleep.

The short time interval between the last meal and the recordings, as well as the diet, assured against any effect due to hypoglycemia. Nevertheless, as an added precaution, the blood sugar levels were checked on all subjects before each recording.

*Methods of analysis.* It was decided to determine the percentage time occupied by the waves of each frequency after the method of Brazier and Finesinger (4). This procedure involved the measuring and tabulating of the time covered by the waves of the various frequencies from 2 to 24 per second. Rhythms higher than these were grouped together and counted as high frequencies (HF). A transparent film was made, marked off in intervals that corresponded to the frequencies to be measured. The records were first examined for artefacts and those areas marked off and not measured. The film was then laid on the strip and the frequency of each wave in one second intervals determined (usually 100 to 200 second intervals were thus measured).

The following rules were adhered to in analyzing the records:

1. A complete wave was one that returned at least more than one half the distance to the base line. Thus, on occasion, a fusion of 2 or more waves of faster frequencies had to be considered as one slow wave. This error was preferred over the one involved in attempting to estimate the probable wave length by extrapolation.

2. The superimposed activity on a wave of 3/sec. or less was measured and recorded rather than the slower wave itself. When no activity appeared on such slow waves, the slow wave was recorded.

3. Jiggles resembling faster activity superimposed on waves of 6 to 13/sec. activity were disregarded. Jiggles occurring between such waves were measured to the nearest wave length (or classified HF).

4. Superimposed activity, other than the exceptions mentioned, were disregarded.

<sup>3</sup> Twelve conscientious objectors of the Glendora Civilian Public Service Camp volunteered for this study and their co-operation was excellent.

The time occupied by the waves of a given frequency was totalled to the nearest 0.2 second, using the calibrated time lines on the record. The figures thus obtained for each frequency were divided by the sum of the time occupied by all the frequencies measured. From these values, percent time frequency distribution curves were plotted. Such histograms were prepared for the electrical activity from the right and left fronto-occipital leads, during "rest" and

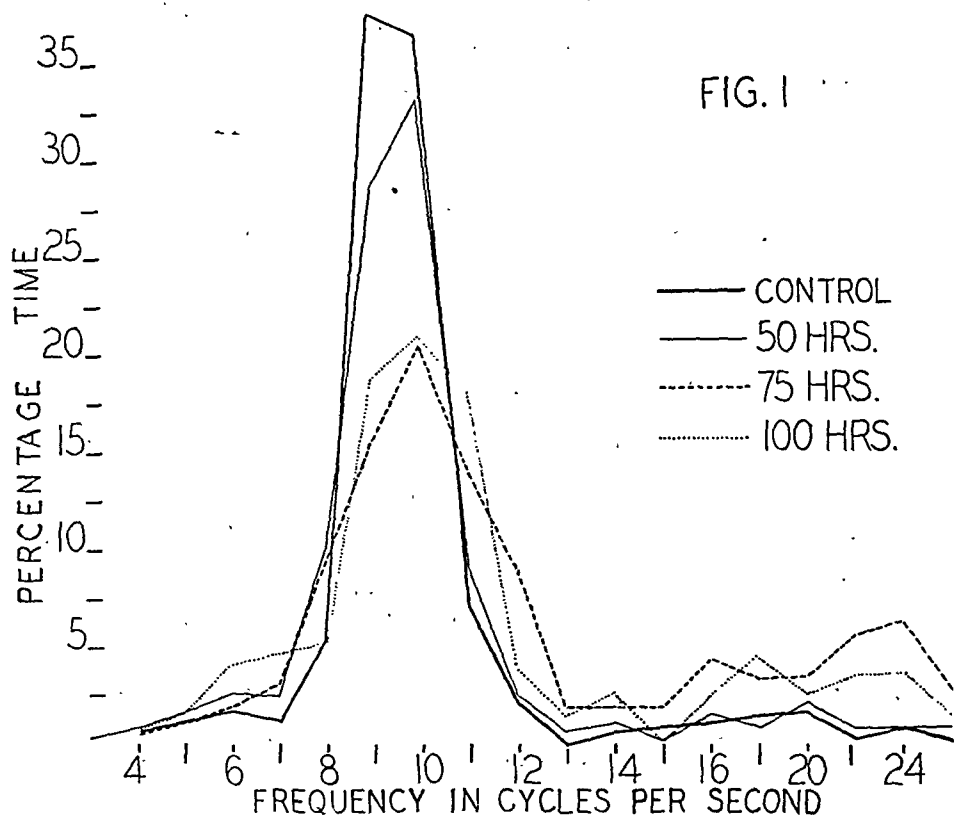


FIG. 1

Fig. 1. The effect of various periods of insomnia on the percentage time frequency distribution. Hours represent duration of insomnia. Values for the various groupings of frequencies given in the text and for columns A in figure 2 are obtained from these curves. For example, the control value for 9/sec. waves = 37 per cent, for 10/sec. wave = 36 per cent. Hence, the combined percentage time for the 9 and 10/sec. waves during that period = 73 per cent. At 75 hours (heavy broken line) 9/sec. waves = 16 per cent, 10/sec. waves = 20 per cent, or a combined percentage time of 36 per cent.

during the performance of a difficult mental arithmetic problem. (Rest is used to describe the condition where the subject was not mentally occupied).

**RESULTS.** 1. *Per cent time frequency curve found during the control days.* The heavy line in figure 1 represents the average resting value found for the 12 subjects during the control days. For an average of 73 per cent of the time the "resting" brain exhibits potential changes at a rate of 9 and 10 per second. Eight per second activity occurs for about 5 per cent, while 11 per second activity averages 7 per cent of the time. Rates below 8, or above 12, occur only in

insignificant amounts when each of the respective frequencies are considered separately. If the time of all the waves of 7 per second and less is totalled the incidence for this group is a little less than 5 per cent of the time. If the rates above 10 per second are grouped arbitrarily into 2 bands, 11 to 17 as one and the 18 per second and higher as another, the amount of time occupied by the former is about 12 per cent, and the latter a little less than 5 per cent. Such groupings are used in figure 2 and the justification for this type of grouping rather than the use of such terms as "alpha, beta and delta" rhythms will be given below.

In 10 of the 12 subjects, the 9 and 10 per second waves occupied the greatest proportion of the time; in the remaining 2, the dominant frequencies were found

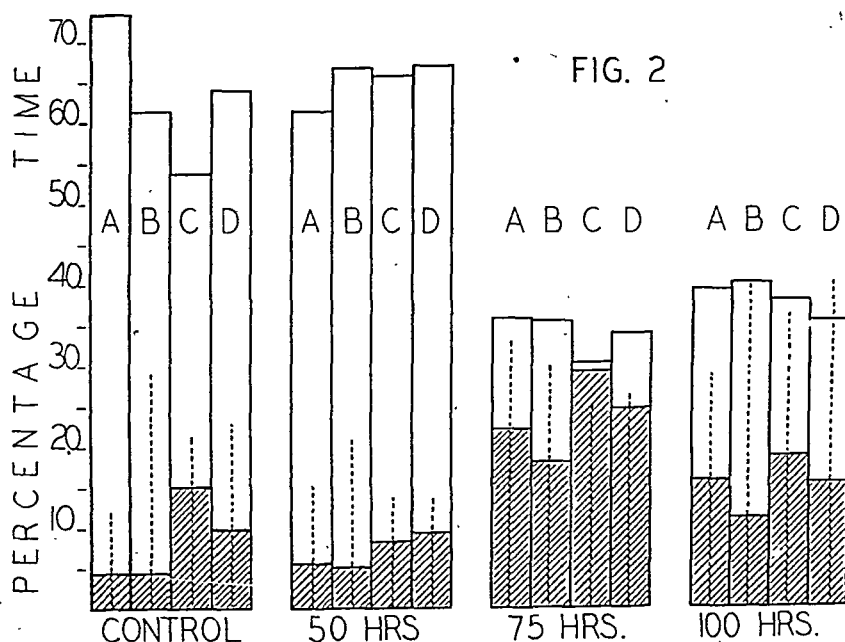


Fig. 2. The effect of a mental multiplication problem on percentage time frequency distribution. Entire length of each column represents the combined times for the 9 and 10/sec. waves; broken lines, the 11 to 17/sec. waves; shaded area, the waves of 18/sec. and higher. A = "resting" value (obtained from fig. 1). B = pre-problem period. C = problem period. D = "recovery".

to be the 10 and 11 per second rhythms. One of these 2, however, showed an abnormal amount of slow activity waves, that is, waves below the 7/sec. In this subject, the time occupied by such waves was 28 per cent (15 per cent of the time being in the waves of 5/sec. and less).

2. *Per cent time frequency curve found during experimental insomnia.* The thin continuous line in figure 1 represents the average resting value found in 4 men at the end of 50 hours. At this stage of sleeplessness the incidence of the 9 and 10 per second activity is depressed and averages 61 per cent of the time, representing a decrease of about 20 per cent when compared with the pooled controls. If comparisons are made for each subject and his corresponding con-

trol value, 3 of the 4 subjects show decreases ranging from 19 to 24 per cent, while the 4th (the subject with the high control level of slow waves) shows little change.

At 50 hours, the incidence of slow activity waves is increased slightly and it now amounts to about 8 per cent. There is also a slight increase in the per cent time found for the 11 to 17 per second waves to a value of about 15 per cent, although relatively little change occurs in the incidence of the rhythms of 18 per second and higher.

After 75 hours of wakefulness (the heavy broken line in fig. 1) the incidence of the 9 and 10 per second wave is markedly reduced. This reduction amounts to 49 per cent. No significant change from that found at 50 hours occurs in the slow activity waves. However, a sharp increase occurs in both the 11 to 17 per second waves and the rhythms of 18 per second and higher. For 33 per cent of the time the potential changes in the brain occur in the former group and for 22 per cent of the time in the latter group, or for a total of over 55 per cent of the time for waves of 11/sec. and higher (compare with 17 per cent during the control days).

At 100 hours (the light dotted line in fig. 1) the picture is essentially the same as that found at 75 hours. The depression in the 9 and 10 per second activity amounts to 46 per cent. There is a slight increase in the slow activity waves; these rhythms occur about 11 per cent of the time. Relatively little further change occurs in either the 11 to 17 group (29 per cent of the time) or the waves of 18 per second and higher (16 per cent of the time).

3. *The effect of mental arithmetic on the per cent time frequency distribution.* a. *During the control days.* Figure 2 represents the changes occurring during mental work<sup>4</sup> in the per cent time of 3 groups of rhythms; the 9 and 10 per second waves (the entire length of the columns); the 11 to 17 per second waves (the broken line running through the center of the columns); and the rhythms of 18 per second and higher (the shaded portion of the columns). The slow activity waves are not represented as only changes at 50 hours are significant and they will be described below.

Four different periods are represented (fig. 2) for each stage of sleeplessness. Column A, in each group represents the "resting" values (subject mentally unoccupied) arrived at from values found (fig. 1) during the control days and at the various intervals of insomnia. Column B is the "pre-problem" period. During this period the subject was told he was to get a mental multiplication problem but had not yet received it. Column C represents the period the subject is given the problem and is mentally solving it. Column D is the "recovery" period, or the period (about a minute) immediately after the subject had given an answer and was at rest again. It may be said that these periods represent different degrees of "attention" or "alertness."

It can be seen that during the control days "mental work" causes a definite change in the distribution of the time spent by the brain in the various grouped

<sup>4</sup> "Mental work" is used as a purely descriptive term. Although it is probable that energy is involved there is, as yet, no good evidence for this.

frequencies, the chief characteristic being the progressive increase in the rate of the electrical rhythm. Going from "rest" to the "pre-problem" period results in a 16 per cent decrease in the 9 and 10 per second rhythms. This decrease is accounted for by an increase in the percent time of the 11 and 17 per second waves. No change occurs in either the slow activity or the waves of 18 per second and higher. Thus, simply "alerting" the subject or bringing him to "attention" increases the rate of the electrical activity of the brain. This acceleration is due to an increase in the incidence of the 11 to 17 per second waves.

Going from the "pre-problem" into the "problem" period results in a further decrease in the incidence of the 9 and 10 per second waves (these are now about 26 per cent below the resting level). The decrease in this rhythm is now accompanied by a decrease in the 11 to 17 per second waves. Both of these reductions are accounted for by a sharp increase in the time occupied by the waves of 18 per second and higher. This latter group shows more than a threefold increase over the resting value. Thus, columns A, B and C show that with increasing alertness or attention the rate of the rhythmic activity of the brain increases progressively up through the spectrum of the frequencies measured.

With the subject at rest again a reversal occurs, and the rate of the potential changes in the brain gradually slows down until it reaches the resting value. This slackening occurs in the opposite direction from the higher frequencies down through the intermediate rates until the resting activity is re-established.

b. *The effect of sleeplessness.* The irregularity of the response is the characteristic effect produced by prolonged wakefulness.

After 50 hours, no clear-cut changes occur in the incidence of the 9 and 10 per second waves. The slight increase in the per cent time of the 11 to 17 per second waves during the pre-problem period is accounted for by a decrease in the incidence of waves of 8 per second and less. The reduction in the incidence of these slow waves also accounts for the slight increase found in the 9 and 10 per second rhythms. During the problem period the waves of 18 per second and higher increase from  $5\frac{1}{2}$  per cent (resting value) to approximately 8 per cent. This is, roughly, only a 30 per cent increase in the incidence of these waves as compared with over a threefold increase during the control days. The recovery period does not show the clear-cut progressive changes that are found during the control days.

In considering the changes at 75 and 100 hours, it is necessary to bear in mind that marked alterations are already produced by sleeplessness. The per cent time occupied by both the 11 to 17 per second waves and the waves of 18 per second and higher have already been sharply increased (columns A). Going from rest into the pre-problem period results in irregular changes in these 2 groups of frequencies. At 75 hours, the incidence of both the 11 to 17 per second waves and the waves of 18 per second and higher decreases, while at 100 hours only the 18 per second waves and higher decrease. Working the problem (columns C), however, at both stages of sleeplessness results in an increase in the waves of 18 per second and higher, but here the increase is only 32 per cent

at 75 hours and 19 per cent at 100 hours (again compare with the threefold increase during the control days). At these stages of wakefulness, the changes in the incidence of the 9 and 10 per second waves are irregular, and although during the recovery period there is a general reduction in the incidence of the waves of 18 per second and higher, the changes are not in the regular and progressive manner typical of the control days.

The control values in figure 2 are the pooled values for all 12 subjects. The direction and the degree of these changes are the same when comparisons are made using each group's own control values.

**DISCUSSION.** We have reported here only the changes in the rate of the electrical activity of the brain. This should not be taken to imply that other types of changes do not occur. At present, the former quantity lends itself most readily to objective analysis. To attempt to evaluate any of the other changes that occur, such as wave form, amplitude, etc., would necessitate the qualification "by our method of analysis or rating." Qualifications of that sort make the most "quantitative" of such studies essentially unrepeatable. The method of analysis used here offers an objective and easily verifiable procedure.

Analysis of the EEG on the basis of the incidence of the various frequencies gives an accurate assessment of the changes in the rhythmic activity of the brain. The per cent time frequency curve found during the control days is identical to, and so confirms, the findings of Brazier and Finesinger (4).

We have made certain arbitrary groupings of the frequencies in this paper, but it has been done for the purpose of simplifying the presentation of data, and no special importance is attached to them. We have not used per cent time alpha as such values would have masked many of the changes that occur within the so-called alpha range of frequencies. The data on the basis of per cent time frequency show that changes in rate, either up or down the spectrum, are not by steps but are continuous.

Changes in the "attention" or "alertness" of the individual, as is produced by performing a mental multiplication problem, result in a progressive increase in the frequency of the potential pattern of the brain. Such changes, produced by various types of mental "activity" and stimulation, have been reported before but not in so quantitative a manner (see 5, 9, 13 for full references). Many workers used procedures that involved counting the number of completed waves per second (18, 12, 7, 16). Such methods, although simpler, give only a rough estimate of the mean frequency. A spectrum so obtained gives a distribution on the basis of waves per second and the extent of the change up through the higher rates of activity are not fully shown.

Mental fatigue, as produced by prolonged wakefulness, also causes progressive increases in the rate of the potential changes in the brain. Barnes and Brieger (1), studying the mental fatigue of a day's work, and using per cent time alpha as a criterion, were unable to detect any changes. The 5 p.m. records of 26 subjects showed no significant differences from those taken at 8 a.m. with the possible exception of susceptibility to deep breathing. They concluded that "it is unlikely that this inherited pattern (the characteristic EEG of the individual) will

be greatly disturbed by mental fatigue." The results reported here indicate that there is little likelihood of any measurable changes occurring during the first 24 hours. Only after 50 hours do marked changes first appear. Under the conditions of these experiments, the brain potentials show a steady increase in frequency up to about 75 hours of experimental insomnia.

Interestingly, the changes produced by wakefulness are in the same direction as those caused by the increased attention or alertness required for the performance of a multiplication problem. That two such different conditions should result in changes in at least identical directions is not strange when one considers that the subject is expending a great deal of mental "effort" or attention in order to remain awake at those times.

Finally, if we superimpose an additional attentional requirement on a brain that is already expending a great deal of effort to stay awake, the regular progressive changes characteristic of the non-fatigued brain are not seen. This may be due, in part, to the marked increases in rate that are already produced by insomnia. From the results here it appears that the capacity of a fatigued brain to further increase its rate of electrical activity in response to the stimulus of an additional problem or additional attention is reduced.

#### SUMMARY

The changes in the potential pattern of the brain have been quantitatively studied during prolonged wakefulness and during mental activity. It was found that:

1. The EEG, quantitatively analyzed on the basis of percent time frequency distribution, affords an accurate objective method of studying the effect of mental activity and of the fatigue of prolonged wakefulness.

2. Changes in the state of attention or alertness as produced by a mental multiplication problem cause an increase in the rate of the electrical activity of the brain. The changes are regular and the extent of the increase parallels the intensity of the mental effort.

3. Increasing periods of experimental insomnia also result in a progressive increase in the rate of the potential changes in the brain.

4. Superimposing an additional mental effort during experimental insomnia results in irregular changes indicating that the capacity of the fatigued brain to further increase its rate of electrical activity in response to such stimulus is reduced.

5. The changes produced by mental activity and by sleeplessness are in the same direction, although under the conditions of these experiments, not of the same magnitude. This is interpreted to indicate that mental effort required either for working a problem or in staying awake during experimental insomnia produces an increase in the rate of the electrical activity of the brain.

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# PHYSICAL CAPACITY OF RATS IN RELATION TO ENERGY AND FAT CONTENT OF THE DIET<sup>1, 2</sup>

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The evaluation of nutritional status in experimental animals has usually been based on criteria such as growth, reproductive activity, lactation and gross pathology. In a series of studies designed to determine the effects of variable fat levels in the diet, we desired to use additional criteria; one such criterion appeared to be provided by a test of fitness for exhausting work.

The military importance of physical fitness in men has led to intensive studies of methods for testing this quality (Keys, 1943; Gallagher and Brouha, 1944; Taylor and Brozek, 1944). The tests in use fall into two major categories: *a*, direct tests, involving the measurement of the duration of exhausting work under standard conditions, and *b*, indirect, involving the measurement of changes in body functions during, at the end of, or in recovery from a work task. In the latter type of measurement, the task may be one which will exhaust all subjects (maximal work), or may be standardized so that all subjects can complete it (submaximal work). The relative merits of these methods have been considered recently by several authors (Gallagher and Brouha, 1944; Taylor and Brozek, 1944; Taylor, 1944).

We wished to use a method for rats which would be more or less comparable with established methods for men, which could be carried out by relatively untrained personnel, and did not involve complicated or expensive apparatus. Indirect methods, such as heart rate measurements, or determinations of lactic acid in blood or urine, as suggested by Crescitelli and Taylor (1944), were compared with the direct (swimming) tests to be described later. They proved unduly difficult, and, in the brief series of measurements made, showed no consistent relation to the results of direct measurement of fitness. Direct methods for man have usually utilized either the bicycle ergometer or the treadmill. The treadmill has been used for rats and dogs (Flinn, 1926; Campos, Cannon *et al.*, 1929). Flinn (1926) found that repeated treadmill runs given to the same animal were accompanied by very considerable increases in performance, indicating that skill and training were important variables. These factors might have been controlled, but the experimental difficulties seemed unduly great. We

<sup>1</sup> The subject matter of this paper has been undertaken in co-operation with the Committee on Food Research of the Quartermaster Food & Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

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accordingly turned to swimming as an alternative possibility; swimming has been widely used to produce fatigue in rats (see for example Miller and Darrow, 1941).

**METHOD.** The specific gravity of the rat was first determined, utilizing a measurement of body volume by displacement. A glass apparatus, diagrammed in figure 1, was devised for this purpose. The apparatus consists of an animal chamber, *A*, large enough to hold a large rat, with a removable top terminating in a small bore tube with a reference mark, *M*. The animal chamber is connected

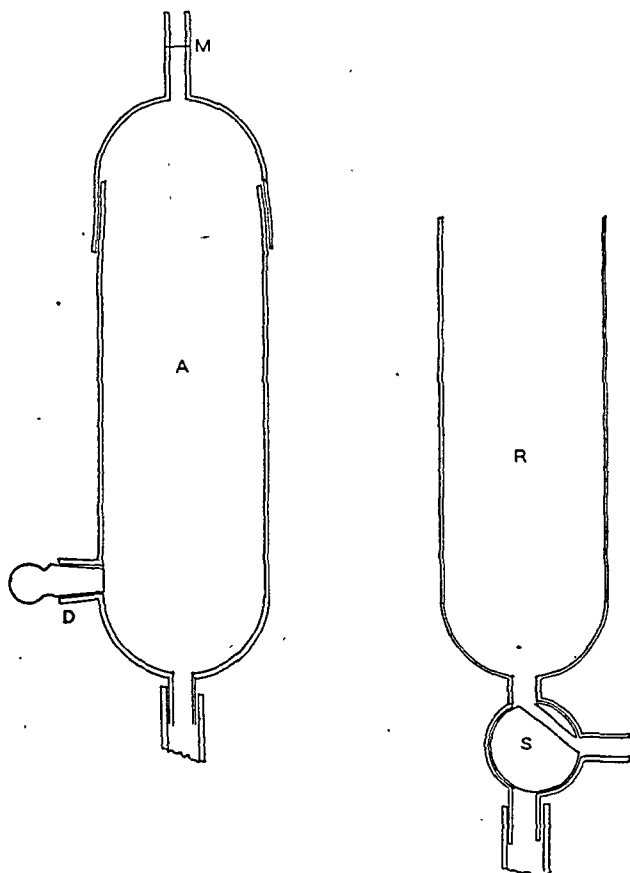


Fig. 1

through heavy walled rubber tubing with a reservoir, *R*, provided with a three-way stopcock, *S*. The animal chamber is provided with an emergency drain, *D*.

For a determination the system was filled with water at 38°C. from the reference mark, *M*, to the stopcock, *S*. Any excess water in *R* was drained off through the stopcock, and the water in *A* then permitted to flow into *R* until sufficient space was available in *A* for the animal. The rat was then weighed and introduced into *A*, the cover was replaced, and water was allowed to flow in rapidly from *R* to reach the mark *M*. The stopcock was closed and the emergency drain at once opened. The water remaining in *R*, representing the

volume of the animal, was drawn off into a tared beaker and weighed. The specific gravity is then simply the quotient of the weight of the animal by the weight of the water displaced. Replicate determinations by this method agreed within 1 per cent.

If the specific gravity of the animal was found to be less than 1.04, brass weights were attached to the thorax with elastic bands to bring the specific gravity within the range 1.04 to 1.06. The animal was then placed in water at least 18 inches deep, at a temperature between 36 and 38°C., and allowed to swim for 2 minutes 50 seconds. At the end of this time the animal was removed from the water for 10 seconds, during which time an additional weight of 2 grams was added. The animal was again allowed to swim for 2 minutes 50 seconds, removed, and another 2 gram weight added. This was continued until the animal was unable to reach the surface during 10 seconds. The physical capacity is taken as the total time in the water, in seconds.

This procedure was developed on the basis of a number of preliminary experiments. The importance of preliminary adjustment of specific gravity appears in the following results. Five female rats, ranging in weight from 191 to 238 grams but of unknown specific gravity were tested on three successive days, without preliminary adjustment of specific gravity. The values obtained ranged from 655 to 2205 seconds, with coefficients of variation for the successive tests of 35.3, 41.8 and 29.7. Five male rats of approximately the same specific gravity were now selected from a number of stock colony animals; the rats selected ranged in weight from 248 to 292 grams, and in specific gravity from 1.045 to 1.053. A similar series of tests was carried out, and the values obtained ranged from 880 to 1350 seconds, with coefficients of variation 13.9, 13.6 and 11.4 on three successive days. This indicated that a part of the variability evident in the first series might be attributed to variations in specific gravity. Accordingly, the same female rats used in the first test, together with others, were tested after preliminary adjustment of specific gravity. Eight rats were used, ranging in weight from 207 to 259 grams, and in specific gravity from 1.030 and 1.060. Specific gravities were adjusted, when necessary, to fall within the range 1.05 to 1.06. In two tests on the same day, the values obtained ranged from 852 to 1431 seconds, with coefficients of variation 15.5 and 11.6. These results indicate that adjustment of specific gravity reduces the variability.

Three difficulties were encountered in our early attempts to use the swimming technique described by Miller and Darrow (1941), in which rats were loaded with a constant weight and allowed to swim to exhaustion. The first difficulty was that of selecting a suitable initial load. A load adequate for small rats permitted large rats to swim for very long periods. It would perhaps be possible to devise a schedule of loads for rats of different sizes, but there is no evident theoretical basis for such a schedule. A similar difficulty was encountered by Gallagher and Brouha (1943) in comparing fitness in boys of different ages.

The second difficulty arose from the fact that rats are able to increase their swimming skill in successive tests. An inexperienced rat, loaded with 10 grams, swam only 15 minutes in the first test, 1 hour in a second test one day later, and

was not exhausted after 2 hours on the third test. The improved performance resulted from elimination of waste motions and the practice of resting on the bottom of the container for several seconds at a time. The third difficulty was that fit rats evidently soon reached a physiological steady state, in which they could continue to swim for very long periods.

This last problem was encountered by Taylor (1941), and solved very successfully in a treadmill test for men. The subject walked on a treadmill operated at a constant speed, but with a continuously and regularly increasing angle of inclination. We adapted this principle to the swimming tests with rats by the method of regular additions of weight described above, and found that this method suffered from none of the three difficulties mentioned. Both small and large rats could be tested, with reproducible results; there was no effect of previous experience, and hence of skill; and there was no evidence that the animals were able to maintain a steady state for prolonged periods. The absence of any effect of learning is demonstrated by a series of three successive tests on five male rats; the average duration of the swims was 1156, 1119, and 1065 seconds on three successive days, showing no increase with experience.

The determination of the end point of a swim offered no serious difficulties. It was observed in the preliminary work that, while rats would frequently submerge voluntarily to swim below the surface, they rarely remained submerged longer than 5 seconds, and almost never longer than 10 seconds. Furthermore, we found that if a rat, obviously exhausted at the end of a swim, was allowed to remain under water much more than 10 seconds, such an animal almost invariably drowned. Consequently, inability to reach the surface within 10 seconds was taken as the endpoint of the swim.

It is not claimed that this method offers an absolute measure of physical fitness, independent of body size. In fact, we have evidence that the duration of swim is to some extent correlated both with body weight and specific gravity. This might be expected from the fact that the successive weights added during the course of the swim are always the same, regardless of the initial weight or specific gravity of the animal. Thus, the small animal is to some extent handicapped by comparison with a large animal. There appears to us, at the moment, no simple way to eliminate this difficulty. Analysis of an extensive body of data has shown that body weight is positively correlated with duration of swim, and that specific gravity is likewise correlated, in both normal and experimental rats (table 1).

It does appear, however, that this method offers a satisfactory means of comparing fitness in rats of similar body size. The method has in particular the advantage that it requires little complicated apparatus, and no special technical skills. An experienced operator can easily test 15 to 20 animals in one day.

**RESULTS.** *A relation of physical capacity to sex and opportunity for exercise.* We have no convincing evidence of any sex difference in physical capacity. In a series of 15 tests involving 5 normal male rats, the mean duration of swim was 1113 seconds. In 24 tests with normal female rats of about the same weight the mean duration was 1132 seconds. The differences between males and females in

table 5, groups Ia and IIa, are negligible except for those fed diet 60a (group Ia). This diet contained no fat, and after weanling rats were fed this diet for six weeks, the males swam for an average of  $805.2 \pm 98.6^3$  seconds, while the time for females was  $512.2 \pm 32.8$ , a difference of  $293 \pm 132$  seconds. Such a difference might arise by chance once in about 35 trials, and consequently cannot be regarded as significant.

In the course of an experiment to be described in more detail below, animals on experimental diets were kept in small individual cages for studies of food consumption. Other animals, on the same diets, were kept in larger cages in groups of 4 or 5 to a cage. An interesting difference which appeared between these

TABLE 1

*Relation of body weight and specific gravity to physical capacity in rats fed experimental diets ad libitum and at a restricted level (12 calories per day) for 6 and 12 weeks after weaning*

GROUP	SEX	MEAN BODY WEIGHT GRAMS	MEAN SPECIFIC GRAVITY	MEAN DURATION OF SWIM SECONDS	COEFFICIENT OF CORRELATION	
					Body weight: duration of swim	Specific gravity: duration of swim
Restricted calories 6 weeks.	Both	46.5	1.0062	342.3	$0.156 \pm 0.13^*$	$0.636 \pm 0.08$
Restricted calories 12 weeks.....	Males	58.6	1.0169	338.8	$0.374 \pm 0.09$	$0.335 \pm 0.085$
Ad libitum, 12 weeks.....	Males	242.6	1.0152	795.5	$0.420 \pm 0.07$	$0.372 \pm 0.08$
Stock diet, ad libitum (normal colony rats).....	Males	144.4	1.0225	800.0	$0.695 \pm 0.12$	$0.565 \pm 0.16$

\* Standard error.

TABLE 2

*Physical capacity of weanling rats kept for 6 and 12 weeks in small and large cages*

SIZE OF CAGE	DURATION OF SWIM IN SECONDS	
	6 weeks	12 weeks
Large.....	$912.9 \pm 36$	$795.5 \pm 24$
Small.....	$624.9 \pm 43$	$710.9 \pm 41$
Difference.....	$288.0 \pm 56$	$84.6 \pm 47$

animals is presented in table 2. At 6 weeks after the beginning of the experiment, when the animals were 9 weeks old, those from the large cages were able to swim much longer than those from the small cages. This may be the result of the fact that the young rats, kept together in large cages, engaged in much more vigorous physical activity than did those kept in small individual cages. The difference had disappeared 6 weeks later, by which time the rats in the large cages had adopted the more sedentary habits of older animals.

*The effect of reduced caloric intake on physical capacity.* These studies form a part of a series of investigations of the rôle of fat in the diet in normal and subnormal nutrition (Deuel *et al.*, 1947, Scheer *et al.*, 1947a, b).

<sup>3</sup> Standard error of the mean.

In a preliminary experiment in this series, male weanling rats were placed on a stock diet and fed at various levels of caloric intake, to determine the minimum level which would support life during 12 weeks. At the end of this time, some of the animals were tested for physical capacity. The results are presented in table 3.

The range of caloric intake extended from nearly normal (normal rats, fed this diet *ad libitum*, attain a weight of about 250 grams in 12 weeks) to a level just sufficient to maintain life during 12 weeks. It appears from table 3 that the physical capacity of rats fed at the same caloric level was less when the rats were placed on the experimental regime at 21 days of age than when they were started at 28 days. This indicates that the observed differences in physical capacity were not due simply to weight differences, since the final weight attained was the same in animals fed at the same level of caloric intake. Table 3 also shows,

TABLE 3

*Effect of undernutrition for 12 weeks on specific gravity, fat content and physical capacity of male rats. Number of determinations in parenthesis*

CALORIES FED PER DAY	NUMBER OF ANIMALS	INITIAL AGE	AVERAGE WEIGHT, GRAMS		SPECIFIC GRAVITY	DURATION OF SWIM
			Initial	Final		
		days				seconds
34.7	9	28	55.4	217	1.04 (4)	935 (2)
27.7	8	28	56.2	180	1.05 (3)	792 (3)
23.1	9	28	60.4	142	1.02 (2)	580 (3)
18.8	9	28	57.5	106	1.01 (2)	457 (4)
14.5	10	28	56.2	72.7	1.00 (4)	333 (3)
24.1	7	21	39.7	142	1.01 (2)	370 (2)
19.1	7	21	42.8	112	0.999 (2)	205 (2)
17.1	7	21	40.8	95.5	0.991 (2)	282 (2)
14.7	7	21	38.7	74.5	0.967 (2)	208 (2)
12.9	7	21	39.5	59.9	0.933 (2)	155 (2)

within any age group, lowered physical capacity at lower levels of caloric intake, but this effect is less striking, for the most part, than is the effect of initial age.

The major series of studies in our program involved three experiments. The animals in each case were fed diets which varied in fat content but provided for a constant intake of protein, vitamins and minerals (table 4). In the first experiment, these diets were fed *ad libitum* to weanling rats. In the second experiment, the same diets were fed at a restricted level of caloric intake (12 calories per day) to weanling rats, litter mates of those used in the first experiment. In the third experiment, rats were fed a stock diet *ad libitum* for 19 weeks, after which they were fed the experimental diets at a restricted level of caloric intake.

At intervals during the course of these experiments, determinations of physical capacity were made. The results of these determinations are presented in table 5. It is apparent that the animals fed restricted amounts of food (groups IIa, b, IIIa, b) had lower physical capacity than did similar animals fed *ad libitum* (group I). A period of *ad libitum* feeding, subsequent to caloric restriction (groups IIc, d, IIIc), restored the values to nearly normal levels.

*Effect of varying levels of fat in the diet on physical capacity.* The data in table 5 have been analyzed statistically as regards the differences between diets of different fat content. The results of this analysis are shown in table 6. This table lists the cases in which the difference of the means of the respective de-

TABLE 4  
*General composition of experimental diets*

DIET NUMBER.....		60A	60B	60C	61	62	63	64	STOCK
Weight per cent	carbohydrate	67.0	67.0	65.0	61.0*	54.0	40.0	12.0	56.4
	protein	25.0	25.0	25.0	27.0	28.0	31.0	37.5	15.1
	fat	0*	0†	0‡	5.0	10.0	20.0	40.0	13.4
Per cent of total calories	carbohydrate	71.1	71.1	67.8	61.5	51.7	34.5	8.6	55.6
	protein	26.5	26.5	26.5	27.2	26.8	26.8	26.9	14.7
	fat	2.4	2.4	5.7	11.3	21.5	38.7	64.5	29.7

\* Contains 1% ethyl laurate.

† Contains 1% methyl linolate.

‡ Contains 2.5% methyl linolate.

TABLE 5

*Physical capacity of rats fed experimental diets varying in fat content. Number of individuals in parentheses*

I. Weanling rats: I<sub>a</sub> fed *ad libitum* for 6 weeks after weaning; I<sub>b</sub> fed *ad libitum* for 12 weeks.

II. Weanling rats: II<sub>a</sub> fed 12 calories per day for 6 weeks; II<sub>b</sub> for 12 weeks; II<sub>c</sub> 12 calories per day for 12 weeks, *ad libitum* for 6 weeks; II<sub>d</sub> fed *ad libitum* for 12 weeks.

III. Young adult rats fed stock diet for 19 weeks: III<sub>a</sub> 24 calories per day for 8 weeks; III<sub>b</sub> 24 calories per day for 8 weeks, 12 calories per day for 4 weeks; III<sub>c</sub>, 24 calories for 8 weeks, 12 calories for 4 weeks, *ad libitum* for 6 weeks.

GROUP	SEX	60A	60B	60C	61	62	63	64	STOCK
I	a	M	805 (5)	692 (5)	1019 (5)	921 (5)	1223 (5)	1143 (5)	855 (4)
			512 (5)	580 (5)	802 (6)	777 (5)	1062 (5)	922 (3)	1025 (4)
	b	M	684 (20)	729 (19)	808 (19)	921 (17)	927 (19)	1026 (15)	912 (19)
II	a	M		259 (5)	317 (5)	383 (5)	310 (5)	340 (5)	343 (4)
				376 (4)	308 (3)	392 (3)	412 (5)	374 (3)	337 (5)
	b	M		327 (17)	338 (17)	299 (19)	350 (21)	374 (22)	325 (23)
	c	M	569 (10)		868 (11)	778 (13)	837 (17)	900 (12)	829 (16)
	d	M	897 (9)		805 (6)	791 (9)	929 (9)	838 (11)	809 (13)
III	a	M	536 (5)		546 (5)	677 (5)	551 (5)	792 (5)	600 (5)
	b	M	276 (9)		271 (9)	272 (11)	290 (13)	319 (19)	351 (18)
	c	M	951 (4)		820 (1)	1050 (1)	994 (5)	1064 (10)	898 (14)

termination exceeds the standard error of the difference by a ratio greater than 2.5 to 1; such differences would arise by chance only once in 100 trials, and consequently may be considered significant. It is apparent from this table, and from table 5, that diets containing no fat (60a, 60b) fed *ad libitum* to weanling rats for 6 or 12 weeks, produce animals with definitely lower physical capacity

than do diets containing 5 to 40 per cent of fat. In the case of group Ib, table 5, it is interesting to note that physical capacity shows a regular increase with the fat content of the diet.

*The relation of body specific gravity to fat content.* Behnke and co-workers (Behnke *et al.*, 1942; Welham and Behnke, 1942) have considered that the variation of specific gravity in men probably depends on variations in the excess fat in the body, and have suggested the use of specific gravity determinations as a rough indication of physical fitness. Rathbun and co-workers (Morales *et al.*, 1945; Rathbun and Pace, 1945) have examined some of Behnke's assumptions

TABLE 6

*Significant differences in physical capacity in rats fed experimental diets.*

For experimental details see text and table 5.

GROUP (SEE TABLE 8)	SEX	DIFFERENCES	RATIO, DIFFERENCE/STANDARD ERROR
Ia	M	60a < 63	2.5
		60b < 61	2.7
		60b < 63	3.7
	F	60a < 61	2.9
		60a < 62	2.6
		60a < 63	4.0
		60a < 64	8.2
		60a < Stock	5.9
		60b < 63	3.5
		60b < 64	7.1
		60b < Stock	5.2
Ib		60a < 62	3.5
		60a < 63	3.5
		60a < 64	4.1
		60a < Stock	2.8
		60b < 62	3.0
		60b < 63	3.0
		60b < 64	3.8
		61 < 64	2.8

experimentally, using guinea pigs, and have found a marked negative correlation between specific gravity and fat content ( $r = -0.962$ ).

Our results do not show such a correlation. In a series of 17 rats fed diets varying in fat content *ad libitum* for 12 weeks after weaning, there was no correlation at all between the specific gravity, measured by our method, and the fat content of the body ( $r = -0.0008$ ). In 21 male rats, fed the same diets at restricted levels of caloric intake, there was likewise no significant correlation ( $r = 0.121$ ). Finally, in 21 male rats fed the diets at restricted levels for 12 weeks followed by 6 weeks of *ad libitum* feeding, a small negative correlation was observed ( $r = -0.428$ ). However, with a sample of this size, such a correlation might have appeared by chance once in 20 samples (Fisher, 1938, p. 214).

Rathbun and Pace (1945) showed that, in normal guinea pigs, there is a constant ratio between the mass of bone and that of other tissues, except fat. It is possible that this is not true for our experimental animals, subjected to various diets and to undernutrition. However, body analyses of these rats (unpublished results) showed no marked differences in the ratio of ash or calcium content to protein, in most cases. A more important variable is probably lung volume. Rathbun and Pace (1945) determined the specific gravity of the eviscerated carcass in their work and consequently eliminated variations due to lung volume. The variations in specific gravity reported in table 3 may possibly be attributed to differences in relative lung volume. The smaller animals, in which lung volume might be expected to be larger, relative to total body volume, have the lower specific gravities. If variations in lung volume should prove to be more important in determining total body specific gravity than are variations in fat content, this might explain the differences between our results and those of Rathbun and Pace (1945).

**DISCUSSION.** The question may well be raised, whether the differences in physical capacity reported here are simply the result of differences in size. Certainly, in most cases, the larger animals had greater physical capacity; growth was greater on diets high in fat than on diets lacking fat (Deuel *et al.*, 1947) and caloric restriction resulted in marked retardation of growth in weanling rats (Scheer *et al.*, 1947a) or loss of weight in adult rats (Scheer *et al.*, 1947b). Lowered physical capacity was also evident in caloric restriction, and rats fed low-fat diets showed subnormal physical capacity. The fact that a correlation exists between physical capacity, measured by our method, and body weight makes it impossible to decide this question without further experimentation.

The differences in physical capacity are not the result of differences in specific gravity, however; there were no significant differences in the specific gravities of animals fed diets containing various levels of fat. The undernourished animals of group IIIa, table 5, had high specific gravities, but reduced physical capacity. Continued undernutrition (IIIb) further reduced the physical capacity, but the specific gravities returned to normal levels.

The usefulness of a determination of total body specific gravity as an index either of fat content or physical fitness, as suggested by Behnke *et al.* (1942), is brought into question by our results. It appears that a correction for lung volume might render the specific gravity determination more useful in this respect.

#### SUMMARY

1. Methods are described for the determination of body specific gravity, and capacity for exhausting work, in rats. The specific gravity determination is based upon determination of body volume by displacement. Physical capacity is determined by measuring the duration of a swim to exhaustion with regularly increasing work load.

2. There was no significant correlation between specific gravity and fat content of the body in the rats studied.

3. There was a small positive correlation between specific gravity and duration of swim, and a similar small correlation between body weight and duration of swim.

4. Physical capacity determined by this method was not influenced by sex or previous swimming experiences. It was influenced by previous opportunity for exercise, and by diet.

5. When diets varying in fat content from 0 to 40 per cent were fed *ad libitum* physical capacity of the animals increased with increasing fat content of the diet.

6. When caloric intake was severely restricted, physical capacity decreased markedly; the level attained was independent of the fat content of the diet.

7. After a period of caloric restriction sufficiently severe to result in death of a considerable proportion of the animals, the physical capacity of the survivors returned to essentially normal levels within 6 weeks after return to *ad libitum* feeding.

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# THERMAL RESPONSES AND EFFICIENCY OF SWEATING WHEN MEN ARE DRESSED IN ARCTIC CLOTHING AND EXPOSED TO EXTREME COLD<sup>1</sup>

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Men experienced in living out of doors in extremely cold weather emphasize the great importance of "under dressing" during periods of muscular activity in order to avoid accumulation of sweat in the clothing (e.g., Siple, 1945). However, it seems to be true that even when the greatest care is taken moisture may accumulate in quantities large enough to endanger survival if exposure is prolonged. Cherry-Gerard (1922) of Scott's polar expedition said:

I never knew before how much of the body's waste comes out through the pores of the skin. "On the most bitter days . . . it seemed that we must be sweating. And all of this sweat, instead of passing away through the porous wool of our clothing and drying off us, froze and accumulated. It passed just away from our flesh and then became ice . . . But when we got into our sleeping bags, if we were fortunate, we became warm enough during the night to thaw this ice: part remained in our clothes, part passed into the skins of our sleeping bags, and soon both were sheets of armour-plate.

Laboratory findings during prolonged tests of uniforms designed for use by the Armed Forces have been consistent with such reports as that of Cherry-Gerard. Furthermore early war experience of the U. S. Army resulted in establishment of special facilities for drying clothing that had been worn in frigid weather either on the ground or in heavy bombardment aircraft at high altitude.

Such observations as these led us to investigate the factors involved in heat balance for men exposed to extreme cold, with particular reference to the part played by the sweating mechanism. The present study is concerned with *a*, amount of sweating; *b*, efficiency of the sweat for body cooling; and *c*, the relationships between sweating on the one hand and skin temperature, internal temperature, and comfort on the other when men are heavily dressed. To obtain a better understanding of the principles involved the environmental temperature and grade of activity have been varied within wide limits.

**METHODS.** The procedures were designed to provide information on amount of sweating, amount of moisture taken up or lost by the clothing, effectiveness

<sup>1</sup> This work was started under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College; it was finished under a contract recommended by the Committee on Quartermaster Problems, between the National Academy of Sciences and the President and Fellows of Harvard College.

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of the sweat for body cooling, energy production, heart rate, internal and skin temperatures and thermal sensation.

Most of the experiments were performed on two young men selected from a larger group because in preliminary trials they did not represent extremes in regard to their sweating response during work and because they were physically fit to perform fairly hard muscular work. The height, weight and surface area of the men were:

<i>Subject</i>	<i>Ht. (cm.)</i>	<i>Weight (kgm.)</i>	<i>Surface Area (sq. m.)</i>
G. S.	176	61-63	1.76
J. S.	178	72-74	1.90

In each experiment the amount of sweating was determined by weighing the subject nude and also fully dressed before and after each exposure. Weighings were to the nearest gram on a Sauter balance. To minimize the known effects of dehydration in reducing sweating (Pitts, Johnson and Consolazio, 1944) the subjects drank 500 cc. of water before the first weighing. The sweat secreted during the actual exposure was computed as (nude weight loss) minus (weight lost through the lungs) minus (weight lost during the dressing and undressing periods).

The weight lost from the lungs included water vapor and excess weight of the  $\text{CO}_2$  expired over the  $\text{O}_2$  inspired. Calculation of the former was based on the assumption that the expired air was at  $91^\circ\text{F}$ . and saturated at a cost of 0.035 grams of body water for each liter of air expired when the ambient temperature was  $20^\circ\text{F}$ . and lower, and at a cost of 0.029 gram at an ambient temperature of  $40^\circ\text{F}$ . The excess weight, in grams, of the  $\text{CO}_2$  expired was calculated assuming an R.Q. of 0.88 by multiplying the oxygen consumption in liters/hr. STP dry by 0.3.

Dressing took about 35 minutes and undressing about 5 minutes. All items except the heavy parkas were put on in a warm room. The final garments were donned in the cool lock outside the cold room while the rectal temperatures and initial skin temperatures were being determined. In an effort to avoid sweating during the dressing period an assistant was provided to aid in the process. The weight loss of the subject during the dressing and undressing periods was calculated to be (nude weight loss) minus (weight loss clothed) minus (moisture gain of clothing). This method of computation was validated by control experiments in which all procedures were the same except that the subject never entered the cold room. These control experiments also provided evidence that the weight change of the clothing itself during dressing and undressing is negligible. After extensive experience with these methods, we estimate that the values obtained for sweating and for total moisture uptake of the clothing during the exposures are usually within 25 grams of the true values.

Energy production in Calories was considered to be 4.9 times oxygen consumption in liters STP dry. During sitting and standing experiments, a continuous record of oxygen consumption and ventilation was obtained by means of a closed circuit apparatus using a 100 liter Tissot gasometer as the oxygen reservoir. During the walking experiments oxygen consumption and ventilation

were measured for five-minute periods half-way through both the first and second hours using an open-circuit apparatus; these measurements checked so closely that the average of the two was considered representative of the entire period of exposure.

The heart rate was determined each half hour by palpation of the radial artery.

Rectal temperature was determined with a clinical thermometer at the beginning and after 60 and 120 minutes of exposure. Skin temperature was determined at 10 locations with copper-constantan thermocouples and a potentiometer. Each couple was read every 15 minutes and the values obtained from individual couples were appropriately weighted when determining mean skin temperature. However, no couple was located on the exposed portion of the

TABLE 1  
*Components of the Arctic Uniform, with weights*

	WEIGHT
	<i>gms.</i>
Shorts, cotton.....	120
Undershirt, 50% wool, 50% cotton.....	370
Drawers, 50% wool, 50% cotton.....	320
Socks, cushion sole.....	80
Shirt, flannel, O.D.....	515
Trousers, wool, O.D.....	1055
Mittens, wool, insert.....	115
Socks, ski.....	250
Parka, pile, M-1944.....	1355
Parka, cotton, M-1944.....	1100
Trousers, cotton, field, M-1944.....	765
Mittens, shell.....	195
Shoes, felt, with felt insoles.....	1310
Total.....	7550

face, which represented about 4 per cent of the total surface of the body. Each item of clothing was weighed to the nearest gram before and after use.

The following standard or experimental U. S. Quartermaster items made up the 17-pound clothing assembly used in this study and hereafter referred to as the "Arctic Uniform" are listed in table 1. This uniform is adequate to protect men who are moderately active at about 0°F. In the conventional units of insulation used for clothing it would be rated at about 3.0' Clos (Siple, 1945).

It is known that pre-dried clothing adsorbs a good deal of moisture from the air when placed in our cold chamber, and theoretically a fraction of the heat given off in the adsorption would be effective for warming the skin.<sup>4</sup> Therefore

<sup>4</sup> Attempts have been made to measure the heat of condensation plus heat of hydration by several workers with results varying between 0.5 and 2.0 Cals./gram for textile yarns (cf. Darling and Belding, 1946).

to reduce adsorption to a reasonable level and to assure some uniformity of adsorption in similar exposures of men in the cold room the clothing was pre-conditioned overnight or longer in a room at 95°F., 50 per cent relative humidity. Initial weight of the individual garments then varied by less than 2 per cent from day to day. Under these conditions it may be assumed that the moisture content of the wool in the garments was about 13 per cent, of the cotton about 9 per cent of dry weight (Wiegerinck, 1940). The data of Darling and Belding (1946) indicate that maximum adsorption in the cold room would probably not exceed 22 per cent for wool and 11 or 12 per cent for cotton, and that even these values would not be applicable for the garments close to the skin because they would be relatively warm. Assuming an average adsorption of 18 per cent as maximum for the 7550 grams, part wool, part cotton Arctic Uniform while worn in the cold room, and assuming an initial moisture content averaging 11 per cent the total adsorption of sweat and environmental moisture might amount to as much as 50 grams during an exposure. Had the clothing been dried in an oven before use adsorption might have reached 130 grams.

The two principal variables, namely, amount of activity and environmental temperature, have been investigated separately.

In studies of the effects of amount of activity the environmental temperature was maintained at 0°F. In separate exposures the subjects sat, stood quietly, walked at five different speeds on the level, and climbed at 3.5 miles per hour up several grades, the steepest of which was 12.0 per cent. (Here the "grade" is the height climbed expressed as a percentage of the distance walked.)

Two separate sets of experiments were performed to determine the effects of environmental temperature. In one set the work performed was constant, a walk at 3.5 miles per hour up a 6.5 per cent grade; in the other, the work was adjusted to give the same rate of sweating at three environmental temperatures so that the effect of temperature on the fate of the sweat might be estimated.

The environmental conditions were these:

Temperature °F.	Relative humidity %
40 ± 2	85 ± 5 (ref. water)
0 ± 2	86 ± 3 (ref. ice)
-20 ± 2	100 (ref. ice)
-40 ± 2	100 (ref. ice)
Wall temperature = ambient temperature within ±2°	
Wind: turbulent, continuous, about 2 m.p.h.	

**RESULTS AND DISCUSSION.** *Inter- and intra-individual variability.* Before selecting the two subjects for intensive studies, experiments were performed with six different men under identical conditions to determine what variability might be encountered among subjects. The most striking result of this study (table 2) was the demonstration that the sweating of two men may differ by as much as 100 per cent. Subject R. W. sweated only 708 grams in two hours under conditions which evoked secretion of 1457 grams by S. C. This great difference in activity of the sweating mechanism of different individuals awaits explanation. As might be expected moisture uptake of the clothing was closely correlated with

sweat production. The variability of the other data is smaller but nevertheless meaningful. Energy production varied by as much as 50 Cals./m<sup>2</sup>/hr. (a 17 per cent difference as compared with an even greater maximum difference of 24 per cent when metabolism was expressed on a unit body weight basis). The low mean skin temperature observed on P. K. was 4°F. less than that observed on any other man and 7°F. less than the highest. Final rectal temperatures differed in the extreme by 1.2°F., a significant amount considering that the average variability for any individual did not exceed 0.3°F.

There is an obvious, though not perfect, inter-correlation among some of these data. A higher rate of sweat secretion was usually accompanied by higher average skin and rectal temperatures, a greater heat production per unit surface area and a larger retention of moisture in the clothing.

TABLE 2

*Inter-individual variability. Average results of two-hour exposures of six subjects at 0°F. They walked at 3.5 miles per hour up a 6.5 per cent grade while dressed in an Arctic Uniform*

SUBJECT	NO. EXPTS.	INITIAL NUDE WT.	SURFACE AREA	SWEAT	MOISTURE UPTAKE OF CLOTHING	ENERGY PRODUCTION	RECTAL TEMPERATURE			FINAL SKIN TEMPERATURE
							Initial	After 1 hr.	Final	
		kgm.	m <sup>2</sup>	gms.	gms.	Cals./m <sup>2</sup> /hr.	°F.	°F.	°F.	°F.
S. C.	4	77.8	1.92	1457	1219	324	99.2	101.7	101.7	90.3
G. S.	7	62.2	1.77	991	797	316	98.7	100.8	100.9	88.8
P. K.	5	76.2	1.95	811	650	277	99.2	100.8	101.0	83.0
J. E.	2	68.5	1.76	742	588	328	100.0	101.6	101.6	87.5
J. S.	7	72.4	1.88	722	599	304	98.5	100.6	100.5	87.9
R. W.	3	65.9	1.79	708	589	288	99.4	100.3	100.5	87.6

The two subjects used for intensive study were dissimilar in their production of sweat, 991 and 722 grams under the conditions of comparison above, but did not represent the extremes found among the six men. Precautions were taken to insure constancy of the state of health and training of these two subjects with results which can best be judged from a simple statistical analysis of the week to week variability in their performance of the same task during the first four months of 1945 (table 3). For each of these subjects sweat secretion and moisture uptake of the clothing, although closely correlated with each other, varied much more than other measurements. Apparently in one experiment out of three under these conditions sweating may be expected to deviate from the average values by as much as 100–150 grams in two hours. Although the subjects were trained to perform this hard work before these experiments were initiated there was some tendency for sweating, heat production and skin temperature (but not rectal temperature) to fall off slightly over this period of time. For example, average sweating of G. S. in the first three as compared with the last three experiments was respectively 1027 and 871 grams and of J. S. was 762 and 706 grams; heat production of G. S. was 322 and 316 Cals./m<sup>2</sup>/hr., and of

J. S. was 309 and 299; and final mean skin temperature of G. S. was 90.4° and 87.2°F., and of J. S. was 90.8° and 86.8°F. The evidence is insufficient to decide whether these were effects of continued training, or of acclimatization to work in this environment, or of thinning of the clothing due to wear.

TABLE 3

*Week-to-week variability. Results of seven two-hour exposures of each of the two principal subjects at 0°F. They walked at 3.5 miles per hour up a 6.5 per cent grade while dressed in an Arctic Uniform*

CATEGORY OF COMPARISON	SUBJECT	RANGE	MEAN	COEFFICIENT OF VARIATION, %*
Sweat (gms./2 hrs.)	J. S.	620-845	722	10.4
	G. S.	818-1139	991	11.9
Moisture uptake of clothing (gms./2 hrs.)	J. S.	523-709	599	12.6
	G. S.	648-944	797	13.6
Energy production (Cals./m <sup>2</sup> /hr.)	J. S.	297-310	304	1.7
	G. S.	295-329	316	3.0
Pulse rate at 2 hrs. (beats/min.)	J. S.	124-132	129	2.6
	G. S.	132-144	138	1.7
Rectal temp. (°F.) initial	J. S.	98.3-98.7	98.5	0.2
	G. S.	98.3-99.0	98.7	0.1
1 hour	J. S.	100.3-100.9	100.6	0.2
	G. S.	100.4-101.1	100.8	0.1
2 hour	J. S.	100.1-101.0	100.6	0.1
	G. S.	100.8-101.2	100.9	0.1
Skin temp. at 2 hours (°F.)	J. S.	85.6-91.0	87.9	2.5
	G. S.	86.6-91.4	88.8	2.0
Initial nude wt. (kgm.)	J. S.	71.1-74.1	72.4	1.5
	G. S.	61.2-62.9	62.2	0.9

$$* \text{Coefficient of variation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100.$$

*Physiological effects of varying activity.* Most of the data obtained in experiments in which the grade of activity was varied while the two subjects wore an Arctic Uniform at 0°F. are summarized in table 4.

Curves have been fitted in figures 1 and 2 to represent sweating as a function of energy production. The curves for the two men are of similar form, with an initial flat portion showing sweat secretion at a rate of 50 to 75 grams for the two hours at levels of energy production below about 175 Cals./m<sup>2</sup>/hr.; this may be regarded as insensible perspiration, not necessarily involving activity of the

sweat glands. As energy production is increased from 175 to about 250 Cals./m<sup>2</sup>./hr. thermal sweating appears, while above 250 and up to about 400 Cals./m<sup>2</sup>/hr. sweating is very nearly a linear function of energy production. It is interesting that despite the fact that G. S. had a smaller surface area he consistently sweated more than J. S.; his rectal temperature also averaged 0.3°F. higher at comparable

TABLE 4  
*Effects of performing various grades of activity for a two-hour period  
in an Arctic Uniform at 0°F.*

NO. EXPTS.	ACTIV- ITY OR SPEED	GRADE	HEAT PRO- DUCTION	VENTI- LATION	SWEAT	MOIS- TURE TAKEN UP BY CLOTH- ING	EFFECTIVE SWEAT	FINAL MEAN SKIN TEMP	RECTAL TEMP.		COMFORT	
									Ini- tial	Final		
Subject G. S. (surface area 1.76 m <sup>2</sup> )												
	<i>m.p.h.</i>	%	<i>Cals./ m<sup>2</sup>/hr.</i>	<i>L./hr. S.T.P.</i>	<i>gms./ 2 hrs.</i>	<i>gms./ 2 hrs.</i>	<i>gms./ 2 hrs.</i>	%	°F.	°F.	°F.	
1	Sit		53	318	8	62	-17		83.6	98.6	97.6	Cold
1	Stand		63	424	71	36	65	92	79.2	99.7	97.8	Cool, feet and hands cold
1	1.29	0	110	519	76	98	36	47	85.0	98.7	98.1	Cool
1	1.80	0	123	578	96	86	62	65	85.5	98.6	99.2	Cool
1	2.25	0	146	727	59	47	44	75	83.0	98.4	99.2	Comfortable
1	3.0	0	167	806	258	188	160	62	84.5	99.0	99.4	Comfortable
3	3.5	0	208	943	253	216	137	54	85.1	99.0	99.7	Comfortable
1	3.5	2.75	238	1088	341	244	196	58	88.5	98.7	100.1	Comfortable
1	3.5	3.25	241	1051	444	309	255	57	87.1	98.9	99.9	Comfortable
1	3.5	3.75	264	1072	515	413	230	45	87.8	98.7	99.9	Warm
1	3.5	4.50	260	1049	574	424	297	52	87.4	98.7	100.3	Warm
7	3.5	6.50	316	1393	991	797	439	44	88.8	98.7	100.9	Hot
2	3.5	9.75	364	1534	1466	1206	569	39	90.6	98.7	101.2	Hot
1	3.5	12.00	426	1787	1892	1535	729	39	88.9	99.0	102.4	Very hot
Subject J. S. (surface area 1.90 m <sup>2</sup> )												
1	Sit		59	528	48	37	44	92	76.1	98.6	98.0	Cold
1	Stand		72	870	67	63	39	58	80.5	98.7	98.0	Cold
1	1.29	0	112	853	91	107	42	46	81.3	98.6	99.1	Cool, hands cold
1	1.80	0	137	972	70	78	40	57	84.0	98.6	99.1	Cool, hands cold
1	2.25	0	145	1098	33	33	29	88	82.5	98.4	98.9	Cool, hands cold
1	3.0	0	173	1366	62	66	39	63	81.9	98.1	99.3	Comfortable, hands cold
3	3.5	0	189	1496	136	134	73	54	82.4	98.4	98.9	Comfortable, hands cold
1	3.5	2.50	216	1694	173	191	67	39	86.7	98.3	99.3	Comfortable
1	3.5	3.00	227	1797	244	225	111	45	85.8	98.2	99.7	Comfortable
1	3.5	3.25	228	1693	306	230	170	56	88.0	98.4	99.9	Warm
1	3.5	4.50	262	1808	446	347	227	51	87.1	98.3	100.1	Warm
7	3.5	6.50	304	2313	722	599	320	44	88.0	98.5	100.5	Hot
2	3.5	9.75	371	2726	1299	1126	472	36	91.1	98.3	101.1	Hot

levels of energy production, but his skin temperature was not shown to be significantly different from that of J. S.

Sweating as a function of metabolism might be expected to follow a sigmoid curve. The characteristic lower curved segment and a long ascending limb are present, but the upper flattened portion associated with maximum capacity of the sweat glands to produce is missing. Data of Robinson, Turrell and Gerking (1945) showing sweating and the values of other factors associated with very

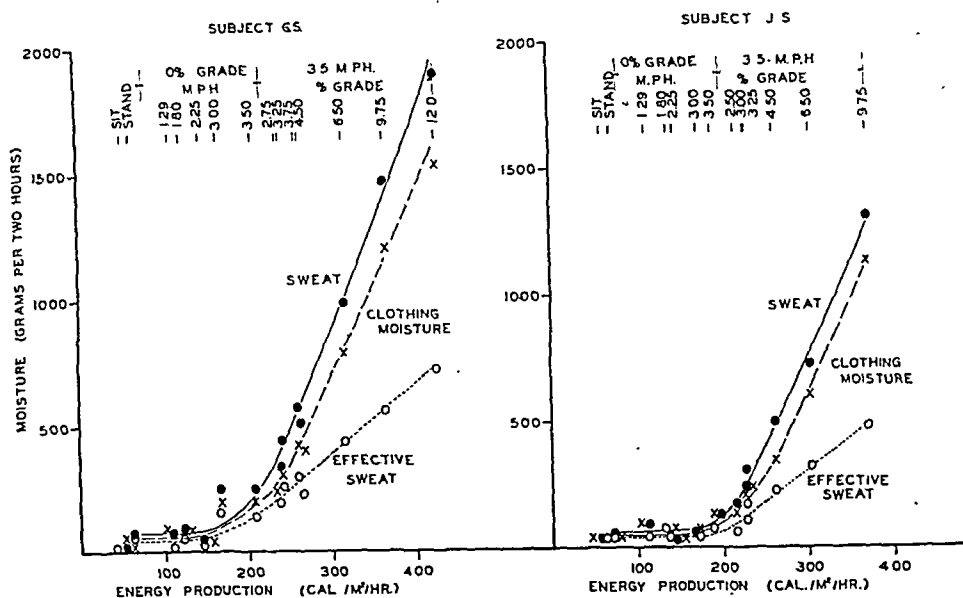


Fig. 1. Total sweat, moisture retention in clothing and effective sweat for cooling plotted as functions of energy production. The subjects sat, stood or walked at various speeds and grades at an environmental temperature of  $0^{\circ}\text{F}$ . while wearing the Arctic Uniform.

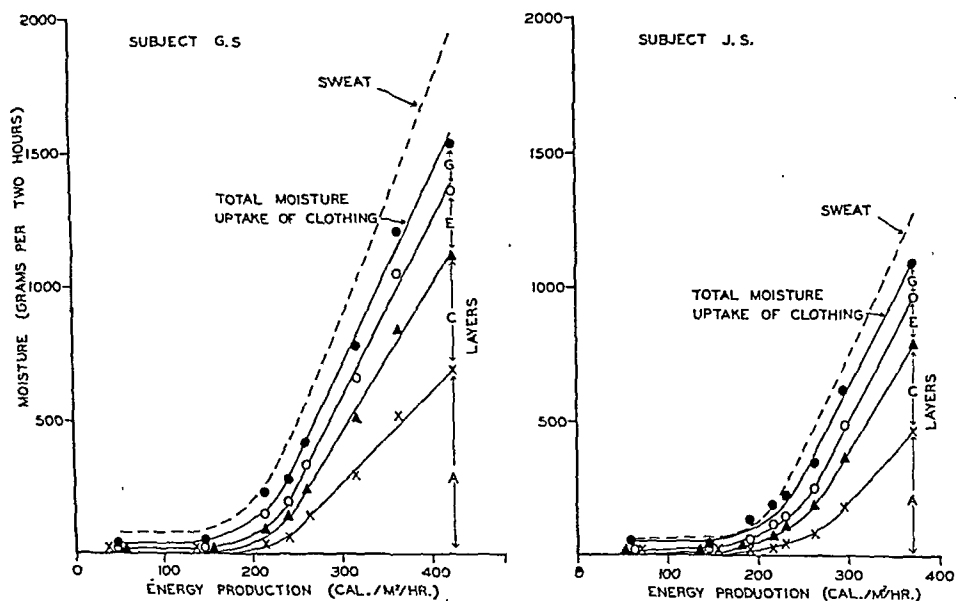


Fig. 2. Total sweat and moisture retention in the layers of clothing plotted as functions of energy production. The subjects sat, stood or walked at various speeds and grades at an environmental temperature of  $0^{\circ}\text{F}$ . while wearing the Arctic Uniform.

severe heat stress in hot environments are compared in table 5 with our data obtained during the most strenuous walk of G. S. at  $0^{\circ}\text{F}$ . to demonstrate that thermal stress as judged from the relatively low final skin and rectal temperatures

in our experiments was insufficient to evoke a maximal output of sweat. It is also interesting that when Robinson et al. observed rates of sweat production similar to ours (bottom of table 5) the skin temperatures of their men were generally higher and rectal temperatures were considerably lower.

Our data show that metabolism and thermal sweating may be linearly related when the clothing and environmental temperature are held constant, but what do they show about the relationship between sweating, skin and rectal temperatures and comfort? These also are correlated in a systematic fashion. With activity such that sweating was at an insensible level, final mean skin temperature was 85°F. or less, final rectal temperature was 99.3°F. or less, and comfort varied from

TABLE 5

*Data selected from Robinson, Turrell and Gerking (1945) for comparison of responses in light clothing in the heat with those of our subject G. S. working very hard in Arctic Clothing at 0°F.*

	ACTIVITY	METABOLISM	SWEAT	RECTAL TEMP.	SKIN TEMP.	PULSE RATE
		<i>Cals./m<sup>2</sup>/hr.</i>	<i>gms./hr.</i>	<i>°F.</i>	<i>°F.</i>	<i>beats/min.</i>
Greatest tolerable stress; Robinson's data, wearing light clothing in the heat	Walking at 3.5 m.p.h. up a 2.5% grade	about 189	about 2000	about 104.7	about 101	about 170
Our data on G. S. in Arctic Clothing; subject "very hot"	Walking at 3.5 m.p.h. up a 12.0% grade	426	946	102.4	89	172
Sweating approximately 946 gm./hr. Mean of 4 expts. of Robinson; light clothing in heat	Walking at 3.5 m.p.h. up a 2.5% grade	about 189	980	99.9	93	105

"cold" in the sitting and standing experiments to "comfortable" at the highest levels of activity which failed to evoke thermal sweating. At metabolic levels between 175 and 250 Cals./m<sup>2</sup>/hr. where sweating is a curvilinear function of metabolism, mean skin temperatures ranged between 82° and 87°F., rectal temperatures between 99.3° and 100.0°F., and the men judged themselves to be "comfortable." When metabolism was raised above 250 Cals./m<sup>2</sup>/hr. mean skin temperatures rose from 88° to 91°F., rectal temperatures from 100.0° to 102.4°F. and comfort ranged from "warm" through "hot" to "very hot". It appears that all of these data are mutually intercorrelated and in the positive sense.

Are thermal comfort sensations to be relied on when men wish to keep sweating at a minimum in the cold? At 0°F. had our men been instructed to maintain

their activity at a level at which they felt "cool but comfortable" thermal sweating would have been avoided. But with other uniforms and at different environmental temperatures if we recommend "underdressing" for each activity to the point of feeling "cool" will thermal sweating likewise be avoided? Also, is the sensation "comfortable" always to be associated with a mean skin temperature between 82° and 87°F. when men are working moderately hard, or do these values apply only when men are working under the particular set of conditions applicable here? We do know that when men are sitting quietly in a temperate environment and are "comfortable" average skin temperature usually lies between 92° and 94°F.

*Physiological effects at different environmental temperatures.* Limited information is available concerning the effects of performing a standard grade of hard work at different temperatures (table 6). The Arctic Uniform that was "hot" for J. S. while working at 0°F. was "comfortable" during the same work at -40°F. When environmental temperature was reduced by 40°F. average

TABLE 6

*Average effects of walking at 3.5 miles per hour up a 6.5 per cent grade in an Arctic Uniform at three environmental temperatures*

Subject J. S.; surface area 1.90 square meters; exposure period 2 hours.

NO. EXPTS.	ROOM TEMP.	METABO- LISM	SWEAT	MOISTURE TAKEN UP BY CLOTHING		EFFECTIVE SWEAT		FINAL SKIN TEMP.	RECTAL TEMP.		COMFORT
									Initial	Final	
	°F.	Cals./m <sup>2</sup> / hr.	gms./2 hr.	gms./2 hr.	% of sweat	gms./2 hr.	% of sweat	°F.	°F.	°F.	
7	0	304	722	599	83	320	44	88	98.5	100.5	hot
1	-20	316	659	607	92	284	43	82	98.8	100.5	warm
2	-40	317	179	190	106	70	39	81	98.5	100.2	comf.

skin temperature decreased from 88° to 81°F. and sweating from 722 to 179 grams in two hours; however, final rectal temperature fell only 0.3°F. At -40°F. the amount of sweat secreted was about equivalent to that secreted at 0°F. at a level of activity involving 100 Cals./m<sup>2</sup>/hr. less energy expenditure (cf. table 4).

When activity was modified in a way to produce approximately equal production of sweat at 3 different environmental temperatures (table 7). the difference in energy production (between 40° and 0°F.) required was again of the order of 100 Cals./m<sup>2</sup>/hr. This study also revealed that at lower environmental temperatures equivalent sweating is accompanied by lower skin temperature and somewhat higher rectal temperature. In other words, rectal temperature, despite the lower environmental temperature associated with greater work, was positively correlated with metabolism, whereas skin temperature was positively correlated with environmental temperature.

*Fate of the sweat; moisture uptake of clothing.* Most of the sweat secreted by the subjects while wearing an Arctic Uniform was retained in their clothing under all conditions of use reported here. Admittedly these were brief exposures, but evidence exists from the results of a five-day continuous exposure of two men

at 0°F. in our cold room and from Arctic experience, that the intermediate and outer layers of clothing accumulate increasing amounts of moisture if given prolonged use where it is not possible to use a fire for drying; on the other hand even when profuse sweating results in wetting of the underclothing during work it rapidly dries afterward.

We seek an explanation both for the observed moisture uptake of the garments in these experiments and the experience with continued use. The amount of evaporation occurring from any surface is primarily dependent on the vapor pressure gradient between the surface and its immediate environment and secondarily a function  $a$ , of air movement (which in turn is dependent on the shape of the exposed surface as well as wind velocity), and  $b$ , the vapor resistance offered

TABLE 7

*Results of single experiments at different temperatures under conditions of activity that resulted in similar sweating*

CATEGORY OF COMPARISON	SWEAT 732 TO 1016 GMS./2 HRS.						SWEAT 403 TO 471 GMS./2 HRS.					
	Subject J. S.			Subject G. S.			Subject J. S.			Subject G. S.		
	+40	0	-20	+40	0	-20	+40	0	-20	+40	0	-20
Room temp. (°F.)												
Speed (m.p.h.).....	3.5	3.5	3.5	3.5	3.5	3.5	3.0	3.5	3.5	2.3	3.5	3.5
Grade (%).....	1.0	6.5	8.0	1.0	6.5	6.5	0	4.5	5.0	0	3.3	4.5
Energy production (Cals./- Kgm./hr.).....	214	316	354	228	312	332	163	262	283	145	241	269
Final mean skin temp. (°F.)..	92.1	86.1	82.6	91.7	85.2	82.7	91.3	87.4	82.9	92.3	85.6	81.6
Final rectal temp. (°F.).....	99.6	100.7	101.3	100.2	100.9	100.6	99.4	100.1	100.1	99.6	99.9	100.2
Sweat (gms./2 hrs.).....	760	732	754	928	968	1016	471	446	403	443	444	471
Per cent of sweat taken up by clothing.....	66	88	96	68	82	87	67	78	71	62	70	89
Per cent of sweat in each layer of clothing.....												
Layer A.....	17	22	24	28	28	28	11	18	-4	17	15	13
Layer C.....	19	27	30	19	23	26	19	24	25	17	20	28
Layer E.....	13	20	23	12	17	19	15	16	23	12	16	25
Layer G.....	17	19	19	9	14	14	22	20	27	16	20	23
Effective sweat (gms./2 hrs.)..	410	317	247	464	417	407	251	228	185	267	252	227
(per cent of sweat).....	58	43	33	50	43	40	53	51	46	60	57	48
Comfort.....	Comf.	Hot	Warm	Warm	Warm	Warm	Comf.	Warm	Warm	Comf.	Comf.	Comf.

by any materials placed between the wet surface and the environment. The principles governing evaporation of moisture from the skin of clothed men in temperate and hot environments have received some attention (e.g., Burton, 1944) but hitherto only casual observations have been made in regard to moisture transfer from the skin in cold environments where the vapor pressure gradient is large and the clothing is thick and heavy. We are aided in our discussion of this topic by the study of Fourt, Fisk, Parrish and Harris (1945) of the transfer of vapor from the wetted "skin" of a cylinder through clothing to the environment, and by the study of Tucker, Goodings and Kitching (1944) of the permeability of textile materials to water vapor.

Here we wish to estimate the amount of evaporation that might be expected with the temperature gradient that existed in most of our experiments. Tucker

et al. have provided physiologists with a convenient formula which may be used for this purpose. Let  $W$  equal grams of water evaporated/m<sup>2</sup>/hr. Let  $F$  be a factor which expresses the vapor transfer in grams/m<sup>2</sup>/hr. for a thickness of dead air equal to 1 cm. when the difference in vapor pressure is 1 mm. Hg. This factor varies with air temperature, from 8.35 at 0°C. to 8.97 at 30°C.; we have arbitrarily selected 8.4 as being applicable for our rough calculations.  $W$  is readily obtained by multiplying  $F$  by  $\Delta V. P.$ , the differences in vapor pressure, and dividing by the thickness of dead air,  $R$ , with which we are concerned:

$$W = \frac{F}{R} \times \Delta V. P.$$

The vapor pressure and moisture-holding capacity of saturated air are plotted as functions of environmental temperature in figure 3.

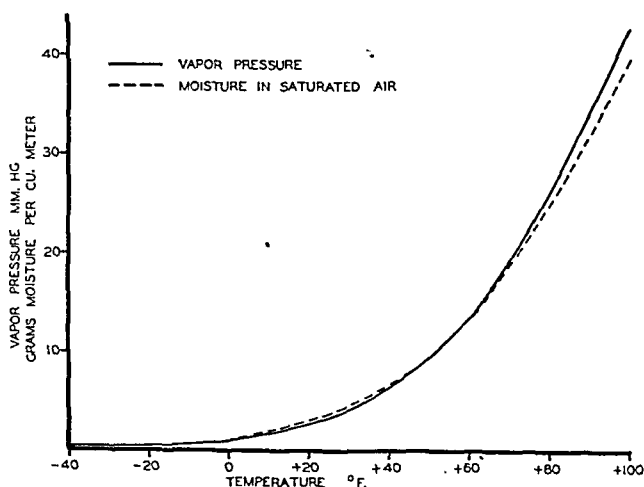


Fig. 3. The relationship of vapor pressure to moisture in saturated air at temperatures ranging from -40°F. to +100°F.

We first consider a hypothetical situation: with a completely wet skin at 93°F. ( $V.P. = 40$  mm. Hg) in an environment at 0°F. with 100 per cent relative humidity ( $V. P. = 1$  mm. Hg) and with a 2-mile per hour wind blowing how much evaporation will occur? Using figures of Burton we have calculated that at this wind velocity the resistance provided by the relatively immobile air close to the skin is equivalent to about 0.23 cm. of dead air<sup>5</sup> (it would be about 0.46 cm. in a room with no perceptible wind). Then

$$W = \frac{8.4}{0.23} \times 39 = 1410 \text{ g/m}^2/\text{hr.}$$

<sup>5</sup> Calculated as follows: the insulation of 1 cm. of dead air is about 1.85 Clos (Burton, 1942); effective insulation with air movement at 2 m.p.h. is about 0.43 Clo (Burton, 1945); therefore 0.43:R::1.85:1, and  $R$  is 0.23,  $R$  being the resistance expressed as an equivalent thickness of dead air.

This is about 3 times the rate of sweat secretion observed in any of our experiments.

If we now interpose the Arctic Uniform in this system the potentialities for evaporation are reduced *a*, because the effective resistance of dead air has been increased and *b*, because the fabrics offer an additional specific resistance to vapor transfer. It will be shown in an accompanying paper that the effective resistance to heat flow by convection and radiation when walking at 3.5 miles per hour is about 1.7 Clo units, and since 1.85 Clos are provided by a layer of dead air 1 cm. thick the effective thickness of dead air in this case is 0.92 cm. We now consider the data of Tucker et al. regarding the specific resistance to moisture transfer of various fabrics; that of underwear is about  $2.2 \times$  the dead air effective for thermal insulation, of worsted serge 2.1, of double pile cloth 1.0 and of cotton poplin 2.3. Let us take the value of 2.0 as the average specific resistance of the uniform; then  $2 \times 0.92 = 1.8$  cm., and

$$W = \frac{8.4}{1.8} \times 39 = 180 \text{ g/m}^2/\text{hr.}$$

Thus the presence of the uniform might be considered to have reduced the potentialities for evaporation from a completely wet skin by more than 80 per cent. Both values for *W* so far obtained are probably too low because the body is made up of a series of cylinders, while the figures of Tucker et al. apply for horizontal plane surfaces from which evaporation occurs at a lower rate.

From the above approximations we might predict that subject J. S., who sweated about 193 grams/m<sup>2</sup>/hr. during a walk up a 6.5 per cent grade at 3.5 m.p.h. would evaporate about all of his sweat to the ambient air when in fact 157 grams/m<sup>2</sup>/hr. (81 per cent of his sweat) were retained in his clothing. Even when sweating occurred at the low rate of 60 grams/m<sup>2</sup>/hr. during a walk on the level as much as 50 grams/m<sup>2</sup>/hr. remained in the clothing.

Why does not the above prediction regarding over-all vapor transfer through the clothing apply? We believe it is because the air temperature in the clothing drops below the dew point and because the clothing provides, from within outward, a series of progressively cooler "screens" upon which condensation readily occurs. We have therefore formulated a hypothesis regarding behaviour of moisture for clothed subjects which takes into consideration the vapor pressure gradient from layer to layer in the system rather than the over-all gradient from skin to ambient air. According to this hypothesis *a*, the vapor pressure of a layer may not exceed that of saturation at the existing temperature of the layer; and *b*, the potential transfer of moisture between contiguous layers is dependent on the vapor pressure difference between the layers.

With the data at hand our hypothesis may be tested in several ways. As a preliminary test we determined with thermocouples the actual temperatures of the layers of clothing over the trunk and arms when subjects were exposed at 0°F. and walking at 3.5 miles per hour. These were found to be about the same regardless of the amount of sweating and the steepness of the climb, and the values obtained have been set down in table 2 together with other data which

make it possible roughly to predict the retention of moisture in the layers of the Arctic Uniform at three levels of sweat production. These predictions are based on the assumption that the resistance to vapor transfer presented by each of the five layers is equal and that the sum is the 1.8 cm. given above.

$$\text{Then } W = \frac{8.4}{0.36} \times \Delta V.P. = \text{grams evaporated from layer/m}^2/\text{hr.}$$

Since one square meter is roughly the surface of the regions covered by these garments (trunk, hips, arms and head) the figures in column 4 of table 8 represent the maximum evaporative transfer to be expected through these garments. At any point in the system moisture in excess of what can be passed to the next

TABLE 8

*Temperatures and assumed maximum vapor pressures in layers of the Arctic Uniform with predicted and actual uptake of moisture by the layers while walking at 3.5 miles per hour.*

*See text for description of method used in making predictions*

LAYER	AVE. TEMP.	V. P. AT SATU- RATION	$\Delta$ V. P. EACH LAYER SATU- RATED	MAX. CAPACITY FOR EVAPORA- TION	SWEAT 538 GMS./M <sup>2</sup> /HR. SUBJECT G. S.		SWEAT 193 GMS./M <sup>2</sup> /HR. SUBJECT J. S.		SWEAT 60 GMS./M <sup>2</sup> /HR. SUBJECT G. S.	
					Actual	Predicted	Actual	Predicted	Actual	Predicted
	°F.	mm. Hg	mm. Hg	gms./m <sup>2</sup> /hr.	gms./m <sup>2</sup> /hr.		gms./m <sup>2</sup> /hr.		gms./m <sup>2</sup> /hr.	
Skin	93	40	16	373	0	165	0	0	0	0
Undershirt	77	24	15	350	168	23	38	0	3	0
Shirt	50	9	5	117	88	233	32	76	4	0
Pile parka	25	4	2	47	122	70	73	70	23	13
Windbreak parka	6	2	1	23	31	24	23	24	16	24
Ambient air	0	1			101	23	24	23	14	23

outer layer will be condensed; it is immediately obvious that much more vapor can be transferred through the inner than through the outer layers.

In general this hypothesis seems to fit the findings in our three sample cases very well, and when there are discrepancies from the prediction there is usually a reasonable explanation. For example, when sweating was heavy (table 8, columns 5 and 6) it was predicted that 165 grams of moisture would be left on the skin. But it is likely that most of this would blot off into the undershirt and apparently that is what occurred. In this same experiment the actual sweat that reached the ambient air exceeded the prediction, but we know from experiments in which impermeable outer clothing was worn that subject G. S. sweats a good deal from the exposed portion of his face, and a fairly large part of the 101 grams that reached the ambient air was probably evaporated from the face. When sweating was moderate (columns 7 and 8) it was predicted that little would be retained in the inner layers, but that the amount in the outer layers

would be about the same as in the experiment in which heavy sweating occurred; such was the case. Also in accordance with expectations, when sweating was light (columns 9 and 10) only the outer layers picked up moisture.

The hypothesis may be tested in at least two other ways. In figures 4 and 5 the final disposition of sweat in the series of experiments in which the grade of work was varied is shown. As heat production and sweating increased under these conditions we expected and found *a*, that loss of sweat to the ambient air reached a maximum first (except for sweat from the face), then *b*, that moisture uptake of the outer and middle layers approached constant maximal values beginning with the outer layer and working inward; also, *c*, that no moisture was retained in the inner layers when sweating was light, but that *d*, these inner layers became the only reservoir for the extra sweat secreted at the harder grades of work.

It is clear from this analysis that under these conditions a man can hope to be rid of his sweat as fast as it is produced only when he is sweating at an insensible level, and then only after adsorption of moisture by the cool outer layers of fabric has proceeded to equilibrium. Actually in several two-hour exposures at the lowest levels of activity moisture adsorption of the outer garments apparently exceeded insensible sweating, suggesting that some moisture was taken from the ambient air.

The explanation for the rapid drying of the inner clothing after a bout of profuse sweating is also implicit in this analysis. Since the underwear stays relatively warm, vapor pressures remain favorable for rapid evaporation of moisture from this layer, much of which will continue to be condensed on the outer layers of clothing.

The hypothesis will also explain the difference in the fate of equivalent amounts of sweat secreted at higher and lower temperatures than 0°F. (table 7). At higher ambient temperatures the flat portion of the vapor pressure curve is avoided with the result that potential vapor pressure differences may be greater between the outermost layers, facilitating evaporation of a substantially greater amount of moisture to the ambient air. However, the vapor pressure gradient between the inner layers may be sufficiently reduced, because of the narrowing of the range of temperatures of the layers of clothing, to increase condensation of moisture in them when heavy sweating occurs.

*Efficiency of sweat for skin cooling.* The effectiveness of sweat for skin cooling depends on its fate. If evaporated from the skin and transferred as vapor to the ambient air we may consider that about 0.58 Calorie were taken from the skin for each gram of sweat, and that the efficiency of the sweat was 100 per cent. But what was the efficiency in these experiments for the sweat that was left in the clothing?

If the hypothesis outlined in the preceding section regarding moisture transfer through clothing is accepted then the efficiency may be readily calculated because the inferences of that hypothesis are clear. Practically speaking we may consider *a*, that most of the sweat remaining in the underwear must have been blotted up from the skin as a result of the fact that the rate of sweating exceeded the capacity for evaporation from the skin at the existing vapor pressure gra-

dient; and *b*, that any sweat retained in the intermediate and outer layers was condensed there after being evaporated from the skin.

In the process of condensation 0.58 Calorie per gram is given up. The principles governing the effectiveness of this heat of condensation for warming the skin are the same as for the effectiveness of electrical heat applied in clothing. A. C. Burton (1941) derived a statement of this effectiveness as follows: Let the insulation worn inside the point of heat supply (condensation) be  $I_1$ , that provided by the clothing and air outside the supply be  $I_2$ . Let the heat supplied at the point of condensation be  $H_1$ ; the temperature of the skin,  $T_s$ ; the temperature at the point where the heat of condensation is supplied,  $T$ ; the temperature of the air,  $T_a$ . Then according to the fundamental equation for heat flow the flow of body heat,  $H$ , up to the region of temperature  $T$  is

$$K(T_s - T) = H \times I_1$$

while for the flow from the region of temperature  $T$  to the ambient air

$$K(T - T_a) = (H + H_1) \times I_2$$

Adding and rearranging

$$K(T_s - T_a) = \left( H + H_1 \frac{I_2}{I_1 + I_2} \right) (I_1 + I_2)$$

Thus the fraction of the heat,  $H$ , which affects the body is  $\frac{I_2}{I_1 + I_2}$ . Then the heat of condensation effective for rewarming the skin equals (total heat of condensation)  $\times$

$$\frac{\text{insulation (clothing + air) outside point of condensation}}{\text{total insulation (clothing + air)}}$$

To take an example, any condensation that occurs two-thirds of the way out from the skin through the insulation is one-third effective for warming the skin. This means that a gram of water evaporated at the skin and recondensed two-thirds of the way out through the insulation has a net effectiveness for skin cooling of two-thirds of a gram.

In the light of the above considerations the net effective sweat has been calculated simply as total sweat minus net condensate effective for body warming. To simplify the computation of the efficiency of condensation the articles of clothing have been separated into four layers, and efficiencies have been assigned as follows:

Layer	Components	Efficiency of condensation
A	Thermocouple harness, cotton shorts, undershirt, underdrawers, cushion-sole socks, wool mitts	100%
C	Wool shirt, wool trousers, ski socks	70%
E	Pile parka	50%
G	Cotton parka, cotton trousers, shell mitts felt shoes	30%

By the simple device of treating moisture picked up in the inner layer, A, as condensate 100 per cent effective in recontributing heat to the skin we obtain a result (indicating 0 per cent efficiency of evaporation) that is consistent with the hypothesis that moisture which remained in the underwear was blotted up from the skin.

Net effective sweat has been calculated for the experiments in which grade of activity was varied and is plotted in figures 1 and 2. Net efficiency for body cooling of the sweat secreted obtained by dividing net effective sweat by total sweat amounted to as much as 65 to 75 per cent at levels of sweat production between 50 and 200 grams in two hours. However, as sweat production increased above about 200 grams the efficiency for cooling declined to less than 40 per cent at the highest levels of production. This relationship between amount of sweating and efficiency of sweating was expected from the previous analysis which showed that when sweating was light a larger fraction reached the ambient air and a larger fraction recondensed in the outer garments where efficiency for rewarming the skin was low.

When sweating was maintained at two levels at each of three different environmental temperatures net efficiency was greatest at 40°F.; intermediate at 0°F., and least at -20°F. Here also the efficiency was less when sweating was heavier, as is shown in this summary of data from table 7:

Temperature °F.	Average sweat 466 grams/two hours	Average sweat 851 grams/two hours
+40	57%	54%
0	54%	43%
-20	47%	37%

It is conceivable that sweat might also accelerate heat loss from the body by increasing the conductivity of the clothing. The importance of this is suggested by the results of a study in which an electrically heated copper foot was dressed with three layers of woolen socks to which known amounts of water had been added. In these experiments evaporation was kept at a minimum by placing an impermeable rubber sock over the outer fabric sock and by keeping the temperature gradient from the skin to the ambient air relatively low. A concentration of moisture equal to 5 per cent of the weight of the socks then increased the conductivity of the sock assembly by about 14 per cent while moisture equal to 10 per cent of the sock weight increased conductivity about 26 per cent. If it is assumed that equivalent concentrations of moisture have about the same effect on conductivity of the Arctic Uniform the effect would be to raise the heat lost through the clothing from 1 to 25 per cent depending on the amount of moisture taken up. On this basis, when our subjects sweated 500 grams per hour at 0°F., heat loss due to increased conductivity was estimated at 13 Calories per hour while effective loss of heat by vaporization of the sweat amounted to about 125 Calories per hour. In most of these short exposures it is probable that heat loss due to moisture in the clothing was unimportant. It would be far more important during rest after a period of sweating, or when the clothing became really wet after several days of exposure.

## SUMMARY AND CONCLUSIONS

Men dressed in an Arctic Uniform have been exposed to several degrees of cold while performing various levels of activity. Data were obtained on sweating, moisture uptake of the clothing, energy expenditure, pulmonary ventilation, skin temperature, rectal temperature and comfort.

The sweating of different subjects while performing the same hard work in the same clothing and at the same temperature varied widely, between about 350 and 725 grams per hour. The subjects who sweated more, generally had higher skin and rectal temperatures as well as higher pulse rates and greater energy production.

The week-to-week variability of values obtained on any one individual was small except as regards sweating, for which the coefficient of variability was about 11 per cent.

During experiments at temperatures between  $40^{\circ}$  and  $-40^{\circ}\text{F}$ . most of the sweat secreted was taken up by the clothing; a larger proportion was taken up at the lower temperatures. When sweating was moderate the uptake was confined to the outer layers, but when profuse the underclothing was soaked. A hypothesis was formulated to explain the principles governing the behavior of the sweat and was found to be tenable for the data. According to this hypothesis the transfer of sweat from the skin does not depend on the over-all difference in vapor pressure between the skin and ambient air; it does depend *a*, on the vapor pressure difference from layer to layer in the system consisting of skin, clothing layers and ambient air, and *b*, on the specific resistance to vapor transfer provided by the fabrics and the air trapped in the clothing. Because the vapor pressure difference between the relatively warm inner layers and the cool outer layers is large there is little tendency to pick up moisture unless sweating is profuse; however, the vapor pressure difference between the cool outer layers and the environment is small with the result that much of the sweat secreted accumulates in the outer garments.

Based on the above hypothesis a method was evolved for determining the net sweating efficiency while a man is dressed. Sweating was shown to be an inefficient way to achieve body cooling when men are heavily dressed in the cold because much of the sweat originally evaporated at the skin is recondensed in the clothing giving back a portion of the heat of condensation to the skin. The amount of heat recontributed was shown to be proportional to a ratio obtained by dividing the amount of insulation lying outside the point of condensation by the total insulation. During walks at  $0^{\circ}\text{F}$ ., the net sweating efficiency was greatest (between 60 and 75 per cent) at low rates of sweat production and least (40 per cent or less) when sweating was profuse.

The data suggest that when it is possible for men to modify their activity to the point of feeling "cool but comfortable" during Arctic exposures sweating and accumulation of moisture in the clothing will be minimal.

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# ANALYSIS OF FACTORS CONCERNED IN MAINTAINING ENERGY BALANCE FOR DRESSED MEN IN EXTREME COLD; EFFECTS OF ACTIVITY ON THE PROTECTIVE VALUE AND COMFORT OF AN ARCTIC UNIFORM<sup>1</sup>

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It has been shown that a large fraction of the sweat produced by heavily clothed men during work in the cold remains in the clothing and is relatively ineffective for skin cooling (Belding, Russell, Darling and Folk, 1947). For example, when a man walked at 3.5 miles per hour up a 6.5 per cent grade at 0°F. sweat was secreted at a rate of about 500 grams per hour, of which 400 were held in the clothing. It was calculated that the net sweat effective for evaporative cooling of the skin was 230 grams per hour, which included the 100 grams that escaped to the environment, plus 130 grams, the calculated effective equivalent of the 400 grams taken up by the clothing. However, despite this low efficiency of cooling per gram of sweat it was concluded that sweating could be a quantitatively important mechanism for heat dissipation; in this example efficiency of cooling by sweat was only 46 per cent, yet 23 per cent of the energy produced by the man was dissipated by this avenue.

One purpose of the present investigation was to determine at various levels of activity not only what fraction of the total energy produced was lost through sweating, but also the part played by the other avenues of energy loss, namely, by convection and radiation from the skin, by warming the inspired air and vaporizing the water in the lungs, and by performing the work of lifting the body. Such information could then be used for evaluating the thermal protection provided by heavy clothing and for revealing factors which may limit its usefulness.

Another purpose was to assess the part played by a suit of Arctic clothing as a thermal barrier under various circumstances of use and to evolve a method of predicting under what conditions this clothing would provide thermal comfort.

Most earlier studies of thermal exchanges of clothed men have been concerned with effects of light clothing on heat exchanges during moderate activity or while resting. One stimulus for those studies came from the air-conditioning

<sup>1</sup> This work was started under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College; it was finished under a contract recommended by the Committee on Quartermaster Problems, between the National Academy of Sciences and the President and Fellows of Harvard College.

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engineers, who wanted to know the range of temperatures and humidities which were comfortable for persons dressed in conventional clothing. Yaglou et al. (1927, 1941) and Houghton, Teague, Miller and Yant (1929) have been active in providing practical answers to this question. Perhaps the most complete analysis of energy exchanges of lightly clothed as compared with nude men has been made at the Pierce Laboratory (Gagge, Winslow and Herrington, 1938; Winslow, Herrington and Gagge, 1938, a, b; and Winslow, Gagge and Herrington, 1939). Using their method of partitional calorimetry, they determined the influence of wall and air temperatures and humidity on storage of body heat and on exchanges of heat due to radiation, convection, and evaporation of sweat.

Gagge, Burton and Bazett (1941) indicated that when the wearer is in a "steady state" the heat flow through his clothing by radiation and convection ( $H_{cl}$ ) may be derived by subtracting evaporative heat loss via the lungs and skin from metabolic energy production; they further showed that if skin and air temperatures are known the insulation provided by clothing may readily be computed. They suggested the "Clo" as a suitable unit of insulation<sup>4</sup>. In those units

$$I_{clo} = \frac{3.09(T_s - T_a)}{H_{cl}} - I_a \quad (1)$$

where  $I_{clo}$  is the insulation in Clo units,  $T_s$  and  $T_a$  are skin and air temperatures in degrees Fahrenheit,  $I_a$  is the insulation in Clo units of the layer of air surrounding the clothing, and  $H_{cl}$  is the heat lost from the body by convection and radiation in Cals./m<sup>2</sup>/hr. We wish to point out that this method of calculation is strictly applicable only when the insulation over all parts of the body is the same. When insulation varies from region to region, as with the Arctic uniform used in this study, the insulation values obtained by the above method may be in error by 10 per cent or more; however, the method has the advantage of being practical and of yielding a useful approximation of insulation value.

These principles and the new unit of insulation were used in wartime for the evaluation of uniforms and sleeping gear designed for military use in cold weather (Burton, 1941; Belding, Darling, Griffin, Robinson and Turrell, 1945). They are employed here, with modifications, to partition the avenues of energy loss of men engaged in various levels of activity while dressed in Arctic clothing as well as to determine the effects of activity on the insulation provided by clothing and to predict the temperature levels for comfort at various activity levels:

<sup>4</sup> One Clo is the protection provided by an ordinary business suit. It will provide comfort for a man while sitting quietly in a room at 70°F. with air movement at 20 ft./min. and humidity less than 50 per cent. Under these comfortable conditions skin temperature,  $T_s$ , averages 92°F., insulation of the air,  $I_a$ , is 0.78 Clo, and metabolic energy production is about 50 Cals./m<sup>2</sup>/hr., of which about 24 per cent, or 12 Cals./m<sup>2</sup>/hr., are lost by evaporation of moisture from the skin and lungs, leaving 38 Cals./m<sup>2</sup>/hr. as the heat lost by radiation and convection,  $H_{cl}$ . Then, substituting in equation (1):

$$I_{clo} = \frac{3.09(92 - 70)}{38} - 0.78 = 1.0$$

**METHODS.** Two subjects, G. S. and J. S., were studied intensively using procedures described by Belding et al. (1947) for determination of sweating, moisture uptake of the clothing, net effective sweating, energy production, pulmonary ventilation, average skin temperature, rectal temperature, and comfort. From these data the factors entering into the energy balance equation were derived. The equation applicable here was

$$M + D = (E_i + A) + W + E_s + H_{cl}, \quad (2)$$

where for convenience all values were expressed in Calories per square meter of body surface per hour.

The factors signified by these symbols are defined and were calculated as follows (cf. Belding, Darling, Griffin, Robinson and Turrell, 1945):

- $M$  = metabolism, and is considered to represent total energy production, both heat and external work. This was calculated from oxygen consumption as previously described (Belding et al., 1947).
- $D$  = heat loss, or debt of the body mass ( $-D$  = heat gain). It was derived (method of Burton, 1945a) by weighting change in mean skin temperature one-third and in rectal temperature two-thirds to determine the change in average body temperature; this value for the change ( $^{\circ}\text{C}.$ ) was then multiplied by the assumed specific heat (0.83) and by body weight (Kgm.) to determine the debt in Calories.
- $E_i + A$  = heat loss as a result of evaporation of water from the lungs ( $E_i$ ) and in warming the inspired air ( $A$ ). Values were obtained by using the nomogram prepared by Belding et al. (1945); applicable assumptions were that the inspired and expired air were saturated with vapor at  $0^{\circ}\text{F}.$  and  $91.5^{\circ}\text{F}.$  respectively (cf. Christie and Loomis, 1933);  $A = 0.65 E_i$  when ambient temperature is  $0^{\circ}\text{F}.$
- $W$  = external work of lifting the body in uphill walking experiments. It was calculated by multiplying kilograms of weight lifted (subject + clothing) by meters of height climbed and by 427 to convert work in kilogram-meters to equivalent Calories.
- $E_s$  = heat loss in vaporization of sweat. This was calculated by multiplying the net effective sweat values obtained as described by Belding et al. (1947) by 0.58 Calories per gram.
- $H_{cl}$  = heat loss through the clothing by radiation and convection (and possibly to a very limited extent by conduction). This was determined by solving equation (2) with  $H_{cl}$  as the only unknown.

All experiments reported here were conducted at  $0^{\circ}\text{F}.$ , with the subjects clothed in the Arctic uniform described in the preceding paper. All experiments lasted for two hours, except one; this was a one-hour run at 6 miles per hour.

**RESULTS AND DISCUSSION.** *Energy exchanges as a function of activity.* Values obtained for the factors entering into energy balance at various levels of activity are given in table 1. The levels of activity represented ranged from sitting quietly (metabolism at 53 Cals./m<sup>2</sup>/hr.) to climbing a 12 per cent grade at 3.5 miles per hour (metabolism at 424 Cals./m<sup>2</sup>/hr.). Data on pulmonary ventilation, rectal temperature changes and net effective sweat, necessary for determination of some of these factors have been presented in table 4 of Belding et al. (1947).

We have data enough on these two subjects to decide in what respects their

responses were similar and different. Both men performed 11 of the 16 activities represented in table 1, activities ranging in intensity from sitting quietly to

TABLE 1  
*Factors in energy balance when performing various grades of activity in an Arctic Uniform at 0°F.*

(For key to symbols see text; values are in Cals./m<sup>2</sup>/hr.)

NO. EXPS.	ACTIVITY OR SPEED	GRADE	M	D*†	E <sub>l</sub> + A*	W	F <sub>s</sub> *	H <sub>cl</sub>	I <sub>clo</sub>	FINAL T <sub>s</sub>
Subject G. S. (Ht. 176 cm.; wt. 62 kgm.; surface area 1.76 m <sup>2</sup> )										
	m.p.h.	%								°F.
1	Sit		53	28	7	0	-3	77	3.1	84
1	Stand		63	45	9	0	11	88	2.6	79
1	1.29	0	110	17	12	0	6	109	2.1	85
1	1.80	0	123	13	13	0	10	113	2.0	86
1	2.25	0	146	10	17	0	7	132	1.6	83
1	3.00	0	167	18	18	0	26	141	1.5	85
3	3.50	0	208	7	21	0	22	172	1.2	85
1	3.50	2.75	238	0	25	15	32	166	1.3	89
1	3.50	3.25	241	-2	24	17	42	156	1.4	88
1	3.50	3.75	264	0	24	20	38	182	1.1	89
1	3.50	4.50	260	-3	24	24	49	160	1.3	89
7	3.50	6.50	316	-8	32	34	72	170	1.2	89
2	3.50	9.75	364	-13	35	52	94	170	1.2	91
1	3.50	12.00	424	-16	41	64	120	183	1.1	90
Subject J. S. (Ht. 178 cm.; wt. 73 kgm.; surface area 1.90 m <sup>2</sup> )										
1	Sit		59	45	11	0	7	86	2.7	83
1	Stand		72	38	18	0	6	86	2.7	85
1	1.29	0	112	33	18	0	6	121	1.8	86
1	1.80	0	137	21	21	0	6	131	1.7	87
1	2.25	0	145	19	23	0	4	137	1.6	87
1	3.00	0	173	8	29	0	6	146	1.5	86
3	3.50	0	189	-3	32	0	11	143	1.5	88
1	3.50	2.50	216	4	35	14	10	161	1.3	88
1	3.50	3.00	229	3	39	17	17	159	1.3	88
1	3.50	3.25	228	3	36	18	26	151	1.4	89
1	3.50	4.50	262	-2	37	25	35	163	1.3	89
7	3.50	6.50	304	-11	48	36	49	160	1.3	89
2	3.50	9.75	371	-21	58	55	72	165	1.3	91

\* Data necessary for deriving these values are presented in the paper by Belding et al. (1947).

† In calculating heat debt an initial mean skin temperature of 90°F. was assumed.

climbing a 9.75 per cent grade. The average values obtained on each man in the eleven experiments appear in table 2.

Since for both men *a*, average energy production was the same on a unit surface area basis; *b*, average final rectal temperature was the same; *c*, average final skin temperature differed by only 1.5°F., and *d*, average heat debt of the body

mass differed by less than 2 Cals./m<sup>2</sup>/hr., it may be concluded that the temperature-regulating centers of both men accomplished about the same adjustment of energy loss to energy production at any one level of activity. In other words the "setting" of the temperature-regulating center, while differing from activity to activity, was at corresponding levels of activity the same for both men.

Total energy loss was about the same for both men at any one activity, and two components of total energy loss were about the same, namely, heat loss through the clothing ( $H_{cl}$ ) and energy loss as work ( $W$ ). However, losses through respiration ( $E_r + A$ ) and through sweating ( $E_s$ ) were quite different for the two men. Subject G. S. had a volume of ventilation which averaged 36 per cent less than J. S. at each activity, and accordingly his loss of heat by this avenue averaged 10.8 Cals./m<sup>2</sup>/hr. less than that of J. S. G. S. compensated for this by sweating more, with the result that he lost 9.6 Cals./m<sup>2</sup>/hr. more by evaporation

TABLE 2

*Average values obtained in the eleven experiments performed by both subjects*

FACTOR IN ENERGY BALANCE	G. S.	J. S.	DIFF.	BODY TEMPERATURE	G. S.	J. S.	DIFF.
	Cals./m <sup>2</sup> /hr.	Cals./m <sup>2</sup> /hr.	Cals./m <sup>2</sup> /hr.		°F.	°F.	°F.
$M$	186.5	186.5	0.0	Final skin T.	85.4	83.9	+1.5
$D$	10.1	11.8	-1.7	Initial rectal T.	98.8	98.4	+0.4
$E_r + A$	19.3	30.1	-10.8	Final rectal T.	99.4	99.4	0.0
$W^*$	31.8	33.5	-1.7				
$E_s$	30.3	20.7	+9.6				
$H_{cl}$	135.3	135.4	-0.1				

\* In 4 uphill walking experiments only.

of sweat than did J. S. It is now evident that the extra sweat secreted by G. S. was secreted in an amount appropriate to compensate for his smaller heat loss via respiration (cf. Belding et al., 1947).

Figure 1, prepared from the data in table 1, shows graphically the interrelations of the factors concerned in energy balance at various levels of energy production. The figure indicates:

a. That for each man heat lost in respiration was a straight line function of metabolism approximately described as follows:

$$\text{for G. S.: } E_r + A = 0.10M \quad \text{for J. S.: } E_r + A = 0.16M$$

b. That the external work done was proportional to total energy production when grade of climb was varied between 0 and 12 per cent at a speed of 3.5 miles per hour; the energy cost of doing one Calorie of external work under these conditions was 3.3 Calories, or expressed in another way the net efficiency of energy expenditure during climbing was 30 per cent.

c. That at rates of energy production up to about 200 Cals./m<sup>2</sup>/hr. effective heat loss by sweating was constant and relatively small in amount (5-10 Cals./-

$\text{m}^2/\text{hr.}$ ) but that at higher levels of production the heat loss through sweating was proportional to the rise above  $200 \text{ Cals./m}^2/\text{hr.}$  and was the most important means of adjusting heat loss.

d. That the combined avenues of convection and radiation ( $H_{cl}$ ) were at all levels of energy production quantitatively the most important for energy loss and that total loss by these avenues increased sharply until energy production reached about  $200 \text{ Cals./m}^2/\text{hr.}$ , then levelled off.

e. That balance of energy production and loss ( $D = 0$ ) occurred only at a production level of about  $250 \text{ Cals./m}^2/\text{hr.}$ ; cooling and warming of the body mass were proportional respectively to the fall or rise in energy production from the level of  $250 \text{ Cals./m}^2/\text{hr.}$ , or

$$\text{for G. S.: } D = 0.15 (250 - M) \quad \text{for J. S.: } D = 0.20 (250 - M)$$

Under these particular experimental conditions the mode of adjustment of energy loss to production was markedly different over the low and high ranges of

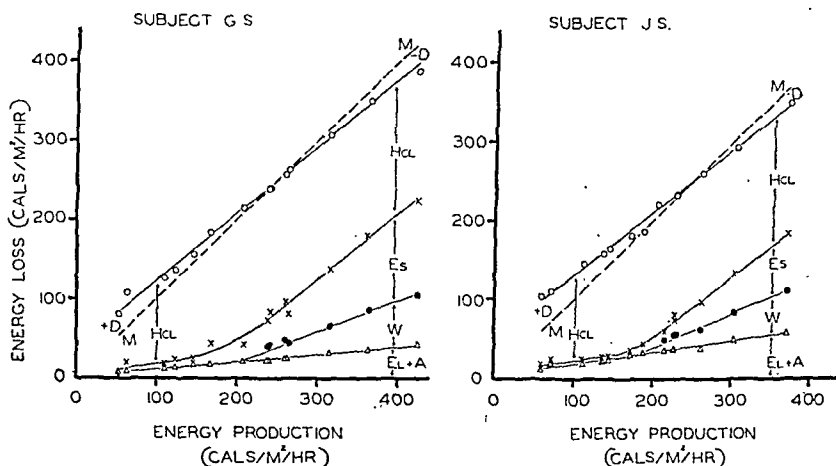


Fig. 1. Part played by various avenues of energy loss at several rates of metabolic energy production. For key to symbols see text.

energy production. Below about  $200 \text{ Cals./m}^2/\text{hr.}$  changes in energy loss by the combination of convection and radiation were most important, whereas at higher energy production levels sweating and external work were most important (table 3).

It is pure coincidence that thermal sweating (as evidenced by rise in  $E_s$  values above the base value of  $5\text{--}10 \text{ Cals./m}^2/\text{hr.}$  indicative of insensible perspiration) and external work both first appeared when energy production rose above about  $200 \text{ Cals./m}^2/\text{hr.}$  Had no climbing been done at levels of energy production higher than  $200 \text{ Cals./m}^2/\text{hr.}$ , we believe that  $E_s$  would have approximated the value of  $E_s + W$  in these experiments. Evidence suggesting that this would be so is presented in table 4, which gives the results of level walking experiments in which energy expenditure was about  $260 \text{ Cals./m}^2/\text{hr.}$

It also seems likely that in downhill walking  $E_s$  would have been reduced by an amount equivalent to the negative work of descent.

*Effects of activity on the insulation value of clothing.* The resistance provided by the clothing against heat losses by convection and radiation, i.e., the Clo value, was more than twice as great when the subjects sat or stood quietly as when they walked moderately fast (table 1).<sup>5</sup> When insulation was plotted as a function of the speed of level progression (fig. 2) incorporating results of additional walking experiments at 4.5 miles per hour and a running experi-

TABLE 3

*Part played by factors in adjusting energy loss to energy production  
(Average of data on both subjects)\**

FACTOR	WITH ENERGY PRODUCTION BELOW 200 CALS./M <sup>2</sup> /HR.	WITH ENERGY PRODUCTION ABOVE 200 CALS./M <sup>2</sup> /HR.
Convection and radiation ( $H_{cl}$ ).....	71%	10%
Sweating ( $E_s$ ).....	16	41
External work ( $W$ ).....	0	36
Respiration ( $E_r + A$ ).....	13	13
	100%	100%

\* Percentage by which the total energy losses were excessive, i.e., caused accumulation of body heat debt, at energy production levels below 250 Cals./m<sup>2</sup>/hr., is represented by

$$\frac{0.18(250 - M)}{M} \times 100;$$

percentage by which energy losses were deficient above 250 Cals./m<sup>2</sup>/hr. is given by the same expression.

TABLE 4

*Comparison of energy losses by evaporation of sweat ( $E_s$ ) and work ( $W$ ) in level and uphill walking experiments which involved similar energy expenditure*

SUBJECT	SPEED	GRADE	M	$E_s$	W	$E_s + W$
	<i>m.p.h.</i>	<i>%</i>				
G. S.	4.5	0.0	261	58	0	58
G. S.	3.5	4.5	260	49	24	73
J. S.	4.5	0.0	261	63	0	63
J. S.	3.5	4.5	262	35	25	60

ment at 6.0 miles per hour it was found that the regression of insulation with speed takes the form of a curve. Starting at about 2.7 Clos when standing, and initially steep, this curve flattens out at 1.2 to 1.3 Clos when speed is 4 miles

<sup>5</sup> Example to show method of calculation using equation (1); when [subject G. S. was sitting quietly  $I_{clo} = \frac{3.09(87-0)}{77} - 0.4 = 3.1$ . The value 87°F. was used for  $T_s$  because it lies midway between 90°F., the assumed initial mean skin temperature, and 84°F., the measured final skin temperature; 0.4 is the insulation of the air with the 2-mile per hour turbulent wind that blows in our cold room (cf. Burton, 1945b for discussion of effects of wind and temperature on  $I_a$ ).

per hour or greater. Although it frequently had been observed that any shift in the position of the body when clothing or sleeping bags were worn in the cold room caused subjects to feel colder, it had not been anticipated that systematic body movements could decrease the effective insulation of clothing to less than half of the value when quiet.

This decrease in effective insulation was so large that reinspection of the values for factors in the heat balance equation seemed desirable. Of the factors determining the value of  $H_{cl}$  in the equation the only one seriously contestable is  $E_s$ , which was derived from the calculated value for net effective sweat using the method described by Belding et al. (1947). What if all the sweat secreted in these experiments were efficient for skin cooling (actually an impossibly favorable situation since so much remained in the clothing)? Then, in a sample case, when G. S. walked at 3.5 miles per hour on the level the insulation value

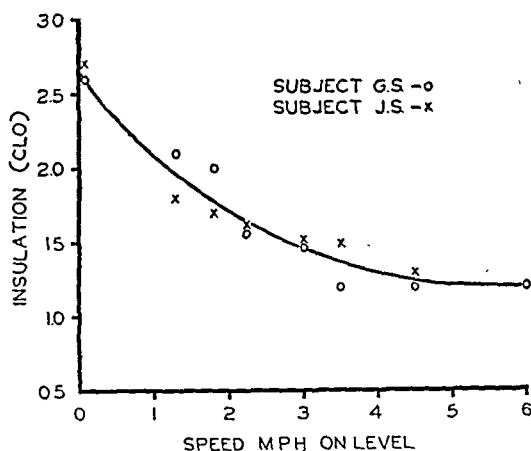


Fig. 2. Insulation provided by Arctic Uniform as a function of speed of forward progression.

would have been calculated as 1.4 Clos instead of the 1.2 Clos arrived at when  $E_s$  was calculated in the way that we have recommended; it is clear that no manipulation of the value for  $E_s$  could possibly raise the insulation value of the clothing in this walking experiment to the value obtained when the man was not exercising. Further calculations show that had the insulation remained at the large value found during quiet standing (2.7 Clos) the subject would have had to sweat over 600 grams in two hours to maintain the same thermal balance even if every gram had been 100 per cent effective for skin cooling; sweating would have had to exceed 1200 grams with the net sweating efficiency which we believe would have been applicable. Yet actually the man only sweated 253 grams in this experiment. The fact that  $I_{clo}$  was 1.2 instead of 2.7 during this walk not only meant a predicted saving of sweat of about 1000 grams in two hours, but also meant that the subject was comfortable instead of excessively warm in this clothing while performing this task. The situation is qualitatively similar for J. S. under the same walking conditions, and for other walking experiments on both subjects. It must be concluded, then, not only that the insulation of this

clothing was reduced during activity, but also that this reduction was appropriate in that it greatly reduced the requirement for sweating.

It would be interesting to learn whether increased radiation or increased convection was responsible for the extra transfer of heat during activity. Potential heat exchanges between the skin and environment were, of course, reduced enormously by the presence of clothing. If no clothing had been worn and skin temperature had been maintained at 85°F. calculations made using constants suggested by Burton (1945b) show that combined losses by radiation and convection would have approximated 650 Cals./m<sup>2</sup>/hr., of which about one-fifth would have been lost by radiation. This may be compared with the actual loss by these combined avenues of about 85 Cals./m<sup>2</sup>/hr. in the sitting and standing experiments and of up to 170 Cals./m<sup>2</sup>/hr. in the walking experiments. Thus it is fair to say that the clothing blocked about 74 to 87 per cent of potential heat loss by radiation and convection.

TABLE 5

	MEAN TEMP. OF SKIN	MEAN TEMP. OF SURFACE OF CLOTHING	GRADIENT
	°F.	°F.	°F.
Sitting quietly.....	84	7	77
Walking at 3.5 m.p.h. up a 6.5 per cent grade.....	89	14	75

Unfortunately it was not feasible to separate the effects of the clothing on losses by radiation and convection. Burton (1943) found that heat loss through kapok-filled air spaces was about 15 per cent greater when the spaces were bounded by plates with blackened inner surfaces than when they were bounded by plates with polished inner reflecting surfaces. This may be taken as an indication that at least 15 per cent of the heat loss through our clothing was by radiation. In attempting explanation of the difference in resistance to heat flow during walking and resting it would seem reasonable to suppose that convective losses were more influenced than radiation losses because such radiation as occurred from layer to layer would presumably be affected only by a changed gradient of temperature through the items of clothing. That such a change in gradient was small and in a direction to decrease transfer by radiation is indicated by the average skin temperatures and surface temperatures of the clothing in sitting and walking experiments (table 5). The latter were obtained both with a radiometer and with thermocouples.

It is relatively easy to see how increased convection losses might occur during walking experiments *a*, as a result of actual infiltration of cold air into the clothing at the garment openings or through the outer windbreak fabrics, or *b*, as a result of mixing the air trapped under the windbreak garments. However, it is not necessary to use the first explanation because when an air-impermeable outer covering (sealed carefully at the ankles, wrists, waist and margins of the face) was substituted for the usual windbreak outer garments effective insulation

during walking fell off fully as much (table 6). In these experiments the subjects walked at 3.5 miles per hour up a 6.5 per cent grade.

With infiltration of air into the clothing ruled out as an explanation for the decrease in effective insulation during walking we turn to an examination of the clothing to determine whether it is reasonable that mixing of air within the clothing should result in a twofold or greater variability in transfer of heat at the same temperature gradient. Certain unpublished data of Paul Siple and his co-workers of the Research and Development Branch, Office of the Quartermaster General, are useful here. These workers measured the actual thickness of the individual garments making up the Arctic Uniform and then determined the girth of wearers as successive layers of the clothing were added over the body. They found that the average total thickness of the fabrics was only about 0.38 inch, but that the total thickness when they were worn on the body averaged about 1.12 inches. The air spaces between the layers must then have had a total thickness of about 0.74 inch. If we assume that these values applied for our subjects what insulation did the fabrics and the air spaces between them provide

TABLE 6

CLOTHING	SUBJECT C. S.		SUBJECT J. S.	
	No. exps.	$I_{clo}$	No. exps.	$I_{clo}$
Arctic Uniform; regular windbreaks...	7	1.2	7	1.3
Arctic Uniform; air-impermeable windbreaks.....	2	1.1	2	1.1

when our subjects sat and stood quietly? When thermal conductivity apparatus has been used with fabrics of moderate density, such as pile fabrics, it has been found (cf. Burton, 1943; also Speakman and Chamberlain, 1930) that the insulation provided by new, clean garments  $a$ , was essentially proportional to thickness,  $b$ , depended very little on the material or weave used, and  $c$ , was about the same as the insulation provided by dead air (about 4.7 Clos per inch). With fabrics of greater density insulation declined somewhat and was greater for wool than for cotton fibres. For this Arctic clothing it seemed fair to figure the fabric insulation at 4.5 Clos per inch, which gave about 1.7 Clos for the thickness of 0.38 inch.

The data of Burton (1943) are also helpful for calculation of the insulation available from the air between fabric layers. He found that air trapped between two stationary, horizontal plates provided 0.31 Clo for the first 0.1 inch of thickness, 0.24 for the second, and 0.11 for the third, and that after thickness reached 0.4 inch the insulation rose no further but totalled about 0.72 Clo. This information was used to calculate the insulation provided by each of the spaces between the garments of our Arctic Uniform. On this basis it was predicted that the total insulation of these air spaces was about 1.4 Clos when the men were not moving, giving a predicted total insulation under these conditions of  $1.7 + 1.4 = 3.1$  Clos. (This value does not include  $I_a$ , the insulation provided

by the relatively inert layer of air against the outside of the outer layer of clothing.)

We can only speculate concerning the effects of forced circulation of the air in the clothing due to frequent and ample body movements. A clue to the effect on insulation of the air trapped in the layers may be gained from the known effects of air movement on  $I_a$ .  $I_a$  is a curvilinear function of wind velocity (Burton, 1945 b) falling sharply from about 0.8 Clo when air movement is equivalent to a straight wind of 35 ft. per minute to 0.1 Clo at very high wind velocities. Since this insulation would theoretically be applicable at each side of each air layer reduction in insulation could be only to 0.2 Clo per space or from an average maximum of 1.4 Clos when a subject was quiet to a minimum of about 0.5 Clo during movement. This alone would result in a reduction of total insulation from 3.1 to 2.2 Clos. However, there is no reason to believe that circulation of air within the garments would be restricted to channels between the garments. The fabrics, except for those in the windbreak items, have a relatively high air permeability, and body movements may well pump air back and forth through them in an amount sufficient to reduce markedly the insulation which they provide.

*Use of a heated cylinder to show effects of movement on insulation.* Because of the indirect methods used in computing these insulation values of clothing from data obtained on men it was decided to check the finding that movement causes a decrease in insulation value of Arctic clothing by making direct determinations on a physical model. Accordingly a thermal conductivity apparatus was constructed in the form of a heated cylinder with dimensions approximating those of a human arm. This cylinder could be driven back and forth to simulate the movements of an arm during walking.

The cylinder was 3.5 inches in diameter and consisted of three separately heated parts, a central or test section 12 inches long, and two end sections 4 inches long which were used as thermal guard rings. A constant surface temperature (92°F.) was maintained on the test section by a Thermistor Electronic Thermoregulator. (This cylinder was similar in many respects to a stationary cylinder designed by the Climatic Research Laboratory of the Office of the Quartermaster General; the thermoregulator was furnished by courtesy of that laboratory.) A watt-hour-meter was used to measure the heat put into the test section to maintain this surface temperature. During operation no heat passed through the ends of the test section because both end (guard ring) sections were maintained at the internal temperature of the test section by carefully adjusting the heat to each guard ring with Variac transformers. Four thermocouples on the partitions between the sections indicated when heat was needed in the guard rings, and the temperature of the cylinder surface was measured at five locations by other thermocouples. Movement was accomplished by suspending the cylinder on the side of a 5.0 inch vertical disc which could be turned at various frequencies. When in motion the top of the cylinder described the circumference of the vertical disc, while the bottom either swung freely, or else was restricted to set amplitudes by the use of padded rings of various sizes.

Sleeves were cut from garments of the Arctic Uniform, drawn over the cylinder and bound tightly to the top guard ring. The fit of the four sleeves (from the underwear, shirt, pile parka and cotton windbreak parka) resembled that of the same clothing on a human arm in that the underwear fitted fairly snugly whereas

the other garments hung loosely with obvious air spaces between layers. Three sets of experiments were conducted, the results of which are summarized in table 7.

The results indicate that nearly as great reductions in insulation are obtainable by moving the model as were observed when men were walking rapidly or running, and that this is true even when infiltration of room air into the garments is prevented.

The measurements made on this heated cylinder seem to confirm the finding on men that the thermal insulation provided by an Arctic Uniform is markedly diminished during movement even though the absolute values obtained on the cylinder do not correspond with those found with the whole uniform on men.

When the fit of garments on the cylinder was tight no such decrease in insulation as a result of motion was observed.

TABLE 7

*Insulation values obtained with sleeves of Arctic Uniform worn over movable copper cylinder*

ARRANGEMENT OF CLOTHING ON CYLINDER	INSULATION (Clo)		
	Cylinder still	Cylinder moving*	Reduction in insul- ation due to moving
Sleeves of regular garments of Arctic Uniform; bottom of sleeves unbound.....	1.7	0.9	47%
Sleeves of regular garments of Arctic Uniform; bottom of windbreak sleeve bound to bottom of cylinder.....	1.9	1.2	37%
Sleeves of inner regular garments of Arctic Uniform; outer sleeve made of rubber dam (air-impermeable) and bound to bottom of cylinder.....	2.1	1.3	38%

\* At 62 cycles per minute, the approximate frequency of arm movement when walking at 3.5 miles per hour; higher Clo values were obtained with lesser amplitudes of movement.

*Prediction of conditions under which the Arctic Uniform will be adequate.* When the insulation value of clothing is known it is possible to predict what environmental temperature will be comfortable at various levels of activity. The relation between ambient temperature for comfort and thermal insulation may be approximately expressed as:

$$T_a = \frac{3.09T_s - (I_{clo} + I_a)(0.75M)}{3.09} \quad (3)$$

Values for  $T_s$ ,  $I_{clo}$ ,  $I_a$  and  $M$  with this Arctic Uniform were assigned on the basis of the following considerations. It has been our experience that the mean skin temperature for comfort in the open does not vary greatly with activity, and may be as low as 90°F.; accordingly 90°F. may be used for  $T_s$ .  $I_a$  may reasonably vary between 0.2 and 0.6 Clo in the open; we select the value of 0.4, which is applicable for a 1.8 to 3.5 miles per hour wind and for our cold room. The average

energy production of the two subjects for each speed of level progression is obtained from table 1, and the average  $I_{clo}$  value at each speed from figure 2. The expression  $0.75M$  is used to approximate the heat losses by radiation and convection ( $H_{cl}$ ); the assumption is that  $E_r + A + E_a$  was 25 per cent of  $M$ ; this is reasonable on the basis of the data on distribution of heat losses in experiments in which our men were comfortable; it also agrees closely with the findings for evaporative heat loss from resting subjects in a comfortable environment (Gephart and DuBois, 1916).

On the basis of these assumptions it is now possible to predict the air temperature for comfort while engaged in various activities. Results of such predictions are plotted in figure 3. The consensus of several subjects regarding comfort at various activities and environmental temperatures in about 100

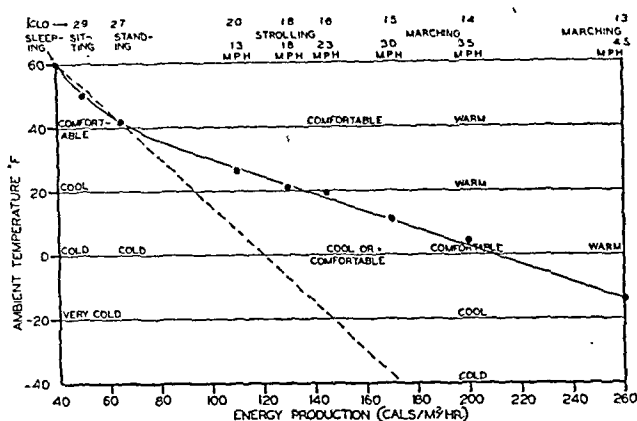


Fig. 3. Ambient temperatures for prolonged comfort in the Arctic Uniform at various activities. Solid line predicts conditions for prolonged comfort on the basis of our data on the insulation provided at various activities. (This insulation is indicated along the top of the figure.) Consensus of actual experience of subjects in two-hour exposures is also given where available. The broken line predicts the conditions for comfort if insulation remained 2.7 Clo regardless of activity. Wind = 2.5 miles per hour.

two-hour exposures in this uniform has been superimposed on this figure, and is found to agree well with the predictions. The range of temperatures within which a man was comfortable for this length of time while performing one activity was apparently somewhere between 20° and 40°F. The range of energy production (during level progression) over which comfort was observed was from 70 to 100 Cals./m²/hr. at any one environmental temperature.

The curve predicting comfort conditions in figure 3 would be displaced toward colder ambients if sunshine were present (cf. Blum, 1945, and Siple, 1945, for estimates of its effect). We would expect it to be displaced toward warmer ambients if part of the energy were expended in external work such as mountain climbing because another avenue of energy loss would be added. On the other hand, when walking down a mountain we would expect it to be displaced toward colder ambients.

The fact that effective insulation decreases when the intensity of activity in-

creases is of practical consequence. It means *a*, that maximum protection is provided when most needed, i.e., when men are idle. It also means *b*, that the range of activities within which a given assembly will provide comfort is larger. For example, in two-hour exposures men were actually comfortable at 40°F. when sitting quietly or when strolling at 2.3 miles per hour, activities involving energy production between 50 and 150 Cals./m<sup>2</sup>/hr., while at 0°F. the men were comfortable while walking at 2.3 miles per hour and at 3.5 miles per hour, activities involving energy production between 150 and 220 Cals./m<sup>2</sup>/hr. If the insulation had been 2.7 Clos at all activity levels the curve showing ambient temperature for comfort as a function of energy production would have been a straight line with a slope so much steeper (see the broken line in fig. 3) that the range of energy production within which comfort could be expected at any one ambient temperature would have been reduced to about half what it actually was, i.e., to between 35 and 50 Cals./m<sup>2</sup>/hr.

TABLE 8

*Predicted requirements for insulation from clothing under conditions of shade and a 2½-mile per hour wind at 3 ambient temperatures*

Actual protection provided by the Arctic Uniform is given; where protection requirements are within 25 per cent of actual protection provided by the Arctic Uniform they appear in italics.

ACTIVITY	<i>M</i>	+40°F.	0°F.	-40°F.	ACTUAL <i>I<sub>clo</sub></i> OF ARCTIC UNIFORM
Sitting.....	50	<i>3.7</i>	7.0	10.3	2.9
Standing.....	60	<i>3.0</i>	5.8	8.5	2.7
Strolling 2.25 m.p.h.....	145	1.0	2.2	3.3	1.6
Level walking 3.5 m.p.h.....	200	0.6	<i>1.5</i>	2.3	1.4
Walking 3.5 m.p.h. up 6.5% grade.....	300	0.4	<i>1.1</i>	1.7	1.3
Level walking 4.5 m.p.h.....	260	0.4	<i>1.0</i>	1.7	1.3

Perhaps clothing could be developed the insulation of which would vary to such an extent that the same clothing would be proper at one environmental temperature whether a man was seated, standing quietly or walking at a brisk pace. Table 8 shows what the insulation should be to provide comfort for men engaged in several different activities at any one of three environmental temperatures. Assumptions were those made in preparation of figure 2 except that where *W* was applicable it was subtracted from 0.75 *M*. Assuming that it would be desirable, how close could we come to providing a uniform which would be reasonably adequate at 40°F. regardless of activity? The present Arctic Uniform would be satisfactory while sitting or standing, but would offer far too much protection if worn buttoned up during hard work. Possibly if all four garments covering the trunk could be opened down the front insulation provided would be reduced to the required 0.4 Clo, but it would be cumbersome to wear this large bulk of clothing unless most of a man's time was being spent quietly, e.g., in a foxhole. At 0°F. the bulk required for protection during sitting and

standing is such that freedom of movement would be hampered. Actually the warmest clothing that we have ever studied provided only about 5 Clos of protection; this means that at below zero temperatures a man must be active most of the time to avoid becoming cold even when wearing the warmest clothing (at night he will be fortunate if he has a sleeping bag that provides 10 Clos of protection). If it were possible to fabricate a clothing assembly which would provide the protection necessary at various activities at one ambient temperature this characteristic should be partially under control of the wearer, that is, it should involve buttoning or some such control. Otherwise if a man found himself in a climate too cold for his clothing he could not warm himself up by exercising, but would have to resort to means of raising energy production which did not increase frequency or amplitude of movement, such as voluntary shivering or isometric contraction of large muscle groups.

Presumably the reason for specifying several separate, relatively thin, loosely fitting items for this Arctic Uniform rather than one or two thick items was to furnish one set of garments that could be used selectively in securing necessary protection while soldiers were engaged in various activities over a wide range of environmental temperatures. These studies of physiological responses have served to indicate additional virtues of this uniform which were perhaps not wholly appreciated by the Arctic experts who helped design it. One was that the complete assembly gave considerably greater warmth for its weight during rest because of the presence of several layers of relatively inert air between the garments. Another was that because such a large part of the potential protection of the assembly depended on insulation provided by inert air between garments the insulation was reduced markedly when body movements "pumped" this air back and forth during exercise. Such a decrease in effective insulation during exercise is considered a desirable feature of any clothing assembly because it extends the usefulness over a broader range of activities at any one ambient temperature, and because it reduces the amount of sweating necessary to eliminate the heat produced at high levels of activity.

#### SUMMARY AND CONCLUSIONS

1. Two subjects have been exposed at 0°F. while dressed in an Arctic Uniform and while engaged in controlled activities involving a wide range of energy production. Measurements were made of oxygen consumption, pulmonary ventilation, body weight loss, moisture uptake of the clothing, and rectal and skin temperatures. The results were used in calculating heat production, body heat debt, sweating, effective heat loss by sweating, evaporative heat loss from the lungs, heat loss from the lungs in warming the air, external work, heat loss through the clothing, and insulation value of the clothing.

2. Activities consisted of sitting, standing and walking at various speeds on the level and uphill. In the lower range of activities (50-70 Cals./m<sup>2</sup>/hr.) the men were cold, in the upper they were hot (energy production 300 to 500 Cals./m<sup>2</sup>/hr.). Under these environmental conditions the principal mode of adjustment of energy loss at energy production levels below 200 Cals./m<sup>2</sup>/hr.

was by modification of convection and radiation losses through the clothing, whereas at higher levels of production, sweating, or sweating and external work were the most important. Heat expended as a result of sweating during level walking about equalled the sum of energy expended in external work and in evaporation of sweat when walking uphill (at a slower speed) with the same energy production.

3. Energy losses exactly equalled energy production only at a metabolic level of about 250 Cals./m<sup>2</sup>/hr. At lower production levels mean body temperature fell and at higher ones it rose.

4. One subject who averaged 36 per cent less volume of respiration than the other at the same level of activity, and had correspondingly smaller heat losses from the lungs, sweated enough more than the other subject to compensate for his smaller heat loss in breathing. Since these two subjects had quite similar energy production (per unit surface area), body heat debt, skin temperature, and rectal temperature, it was concluded that the "settings" of the temperature regulating centers of these two men when engaging in similar activities were quite similar.

5. The insulation provided by the clothing was a curvilinear function of speed of level progression, was 2.7 Clos when the subjects were standing quietly, 1.6 when walking at 2.25 miles per hour, and 1.2 when running at 6 miles per hour. Convection currents set up between the layers of clothing and through the relatively air-permeable garments lying under the windbreak layer were held to be responsible for this decrease in insulation.

6. Predictions were made concerning the ambient temperatures for comfort in this uniform at various grades of activity and were shown to agree with experience of a number of subjects. Consideration was also given to the desirability and possibility of designing clothing which would maintain comfort at some one temperature regardless of activity.

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# THE RELATIONSHIP BETWEEN TRAUMATIC SHOCK AND THE RELEASE OF ADENYLIC ACID COMPOUNDS

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Ever since the observation of Drury and Szent-Györgyi (1, 2) that adenosine compounds, free or phosphorylated, exert a marked depression on the arterial blood pressure, interest has been directed toward this group of compounds as possible factors in traumatic shock, particularly that resulting from crushing injuries. From the work of Embden and his group (3) and of Fiske and Subbarow (4) it is well established that skeletal muscle is rich in phosphorylated adenosine compounds. The possibility of adenylic acid or adenylyl-pyrophosphate being responsible for traumatic shock has been suggested by many authors since 1930 (5-8). Release of adenosine-like substances into the blood as the result of trauma has also been reported (6, 7).

The determination of adenosine compounds in dilute solution has so far been based on its pharmacological effect on smooth muscle. This technique, although sensitive, is not only tedious but also rather nonspecific. By means of differential ultraviolet spectroscopy carried out with specific deaminases (9), it has been possible to determine adenosine compounds in small samples of plasma in concentrations as low as 0.5 mgm. per cent or less.

Using these specific analytical procedures, the plasma levels of several of the pure adenosine compounds have been correlated with the decrease in blood pressure resulting from their administration. Subsequently, the plasma of shocked animals was tested to establish whether or not there was a sufficient concentration of adenosine compounds to explain the hypotension. In addition, since deaminated adenosine compounds (inosine and inosine phosphates) are pharmacologically inert (7)<sup>2</sup>, enzymes capable of deaminating the purine depressor substances were tested *in vivo* for their ability to overcome the depressor action of injected adenosine derivatives or to elevate the blood pressure of shocked animals.

**METHODS AND PROCEDURE.** Arterial or venous blood samples (2 to 4 ml.) were heparinized and the plasma was analyzed for adenosine compounds by optical methods described elsewhere (9, 10). The free and phosphorylated adenosine derivatives were usually measured together, since they all have depressor activity.

Both rabbits and dogs were used for the shock experiments. For rabbits, spinal anesthesia proved more satisfactory than ether and was used in the later

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<sup>2</sup> The hydrolysis products of adenosine (adenine and ribose) are also inactive.

experiments. This was accomplished either by spinal section in the lower lumbar region or by intraspinal injection of novocaine in the same area. The animals thus prepared remained apparently unaffected while the trauma was being applied (500 to 800 blows with a mallet over the complete area of 1 extremity) and for the first 4 or 5 minutes after traumatization. Soon thereafter the animals became weak and drowsy. If, after administering the trauma, the systolic pressure was 85 mm. mercury or higher the traumatized extremity was subjected to additional mechanical damage until the arterial pressure fell below 85 mm. The damaged extremity was then bandaged in tape to prevent undue swelling.

For the shock experiments with dogs, general ether anesthesia was used and both legs were usually subjected to mechanical trauma in order to cause sufficient decrease in the arterial blood pressure.

**RESULTS.** *Action of adenylic acid on blood pressure*<sup>3</sup>. Dogs were infused through the ear vein with a solution of adenylic acid (3-adenylic acid or 5-adenylic acid) and the arterial blood pressure was measured at intervals before, during, and after the infusion by direct femoral puncture (under local anesthesia). Blood samples were drawn from the jugular vein of the side opposite to that in which the adenylic acid solution was infused.

Table 1 summarizes the results obtained in an experiment in which 750 mgm. of 3-adenylic acid<sup>4</sup> (yeast adenylic acid) in 125 ml. saline were infused at a rate of about 35 mgm. per minute. The data indicate that the presence of 2 to 5 mgm. per cent of 3-adenylic acid in the plasma of the general circulation results in a moderate to severe decrease in the arterial blood pressure. The steep rise and fall in the concentration seems of interest. The level in the plasma fell from 80 mgm. per cent during the administration to 5 mgm. per cent, 8 minutes after the end of the infusion. This fall in the concentration of 3-adenylic acid in the circulating plasma can not be explained solely by diffusion into the extracellular spaces because of the low levels reached, but must be attributed to penetration into the cells and/or destruction.

In table 2 are summarized the results of an analogous experiment in which 250 mgm. of 5-adenylic acid<sup>5</sup> (muscle adenylic acid) in 70 ml. saline were injected at a rate of 30 mgm. per minute.

The 5-adenylic acid produced a surprisingly small increase in the plasma level of adenylic acid, even during the period of injection. The discrepancies between the plasma levels produced by the 3- and 5-adenylic acids are much greater than the 3-fold difference in the amounts of the two acids injected. The blood pressure was definitely lowered during the period of injection but returned almost to normal within 4 minutes after the end of the infusion. Gillespie (12) reported that 5-adenylic acid is a more powerful depressor than 3-adenylic acid. It seems likely that 5-adenylic acid may pass much more readily than 3-adenylic acid into

<sup>3</sup> These experiments were performed in co-operation with Drs. C. R. Houck and F. N. Craig of New York University, College of Medicine, to whom we express our sincere thanks.

<sup>4</sup> B. L. Lemke Co., New York, N. Y.

<sup>5</sup> Armour and Co., Chicago, Ill.

the cells. Moreover, various tissues, particularly skeletal muscle, contain enzymes capable of deaminating 5-adenylic acid.

*Action of polyphosphorylated 5-adenylic acids on blood pressure.* Adenosine triphosphate is generally considered to be a much more potent substance pharmacologically than adenylic acid (adenosine monophosphate). Thus, Fleisch and Weger (13) report that for the cat and dog adenosine triphosphate is a

TABLE 1

*Infusion of 3-adenylic acid*

BLOOD SAMPLE NO.	ELAPSED TIME	ARTERIAL PRESSURE*	PLASMA ADENYLIC ACID
	min.	mm. Hg	mgm. %
1	0		0.2
	23	140-142	
2	26		0.3
	41	Start of 3-adenylic acid infusion	
3	46		80.0
	60	47	
	60½	End of infusion (750 mgm.)	
	66	58	
4	68		4.7
	86	104	
5	95		2.0
6	119		1.0

\*Measured by Dr. C. R. Houck.

TABLE 2

*Infusion of 5-adenylic acid*

BLOOD SAMPLE NO.	ELAPSED TIME	ARTERIAL PRESSURE*	PLASMA ADENYLIC ACID
	min.	mm. Hg	mgm. %
1	0	120-130	
(venous)	1		0.2
	20	126-130	
2	22		<0.2
(venous)	29	Start of 5-adenylic acid infusion	
	33	128	
	34	90	
3	34½		0.9
(venous)	35	84-85	
	37	End of infusion (250 mgm.)	
4	39		0.8
(venous)	41	118-122	
	47		0.25
5			
(arterial)	87		<0.2
6			
(arterial)			

\* Measured by Dr. C. R. Houck.

hundred times more effective than adenylic acid as a dilator of the peripheral vessels.

Using the rabbit, we have failed to find a similar difference in effect on the arterial tension. Equimolar amounts of adenylic acid, adenosine diphosphate and adenosine triphosphate were separately injected intravenously into a rabbit. Administration of the equivalent of 0.2 to 0.4 mgm. of adenylic acid resulted in essentially the same depressor response with each of the 3 compounds (decrease of 15 to 30 mm.).

The effect of adenosine deaminase and phosphatase on adenosine compounds *in vivo*. An anesthetized rabbit was infused through the femoral artery with a saline solution of 25 mgm. of adenosine triphosphate<sup>6</sup> (calculated as adenylic acid) at a rate of 4 mgm. per minute. About 2 minutes after the infusion was started 4 ml. of a mixture of adenosine deaminase, alkaline phosphatase, and adenylyl pyrophosphatase were injected intravenously, without interrupting the infusion of adenylyl pyrophosphate. (*In vitro* the adenylyl pyrophosphatase would rapidly split off the two pyrophosphate groups of adenosine triphosphate, the alkaline phosphatase would remove the stable phosphate group, and the adenosine thus liberated would be rapidly deaminated by the adenosine deaminase.) The arterial blood pressure at the start of the infusion was 80 mm. and 2 minutes later, just before the injection of the deaminase mixture, it had fallen to 52 mm., and seemed to be falling steadily. After the injection of the deaminase mixture the fall was not only arrested but the pressure increased promptly to 65 or 70 mm. and then remained practically constant at this level over a 4-minute period. When the infusion of adenylyl pyrophosphate was stopped the

TABLE 3  
*Destruction of adenosine triphosphate in rabbit plasma*

INCUBATION	MGm. % ADENOSINE TRIPHOSPHATE REMAINING AFTER INCUBATION	
	Plasma from normal rabbits	Plasma from enzyme-injected rabbits
<i>min.</i>		
0	14.2	13.5
5	15.0	10.2
15	13.9	2.8

blood pressure returned to 80 mm. In separate experiments it was established that the mixture of enzymes had no influence on the blood pressure under normal conditions, and that the efficacy of the enzyme mixture was abolished by boiling at a neutral reaction for 1 minute. It seems obvious that the deaminating enzyme system was effective *in vitro*.

*Retention of injected deaminase-phosphatase mixture in plasma.* Plasma obtained from an animal about 1 hour after an intravenous injection of a mixture of deaminase-phosphatase enzymes, was analyzed for these enzymes as follows: Samples were incubated with adenosine triphosphate (15  $\mu$ g. as adenylic acid per ml.), deproteinized, and assayed for remaining adenosine triphosphate (table 3). Although normal plasma is devoid of adenosine triphosphate-destroying enzymes, the plasma from the animal injected with the deaminase-phosphatase mixture apparently still contained effective amounts of such enzymes 1 hour after injection.

*Adenosine compounds in the plasma of shocked animals.* It appears (table 4) that there were, in several cases, slight but distinct increases in the concentration of adenosine compounds in the venous plasma coming from the traumatized extremities of shocked animals. The highest levels of adenosine derivatives in

<sup>6</sup> Prepared in this laboratory.

the plasma from the injured limb were observed early after the traumatization. In some cases no increases were found although the blood pressure was greatly diminished. Since the concentration of adenosine derivatives in the venous plasma from the traumatized tissue was never very high, its contribution to the level in the systemic circulation would necessarily be small. It is, therefore, not surprising that in no instance was an increase in the systemic venous plasma concentration observed. The presence of adenosine in the arterial plasma in a single instance (dog C) is difficult to interpret, and may be an artifact, since in no other case were adenosine derivatives detected in the peripheral circulation.

TABLE 4

*The concentration of adenosine compounds in the plasma of animals in traumatic shock*

ANIMAL	CONDITION	SOURCE OF BLOOD	PLASMA ADENOSINE COM- POUNDS	ANIMAL	CONDI- TION	SOURCE OF BLOOD	PLASMA ADENOSINE COM- POUNDS
			<i>mgm. %</i>				<i>mgm. %</i>
Dog A*	Control	Systemic vein	<0.2	Rabbit A	Shock	Traum. vein	0.4
	Shock 2'	Traum. vein	0.6		Shock	Systemic vein	<0.2
	Shock 60'	Traum. vein	<0.2		Shock	Artery	<0.2
	Shock 60'	Systemic vein	<0.2				
	Shock 60'	Artery	<0.2				
Dog B	Control	Systemic vein	<0.2	Rabbit B	Shock	Traum. vein	0.9
	Shock	Traum. vein	<0.2		Shock	Systemic vein	<0.2
	Shock	Systemic vein	<0.2				
	Shock	Artery	<0.2				
Dog C	Control	Systemic vein	0.25	Rabbit C	Shock	Traum. vein	0.8
	Shock 30'	Traum. vein	0.70			Systemic vein	<0.2
	Shock 60'	Traum. vein	0.55				
	Shock 60'	Artery	(0.85)				
	<i>Exitus</i>	Systemic vein	0.20				

"Shock" indicates arterial pressure under 85 mm. Hg. "Traum. vein" indicates blood from femoral vein draining the injured leg.

\* Blood samples from this animal were obtained through the courtesy of Drs. R. A. Phillips and R. M. Archibald of the Hospital of the Rockefeller Institute for Medical Research.

As further evidence that adenosine compounds may be so low in the blood of shocked animals as to be without significance, it was found that the intravenous injection of adenosine deaminase plus phosphatase into animals in traumatic shock had no effect on the blood pressure.

It seems, therefore, doubtful that the release of adenylic acid compounds plays a primary rôle in the etiology of traumatic shock. It is, of course, difficult to rule out the possibility that the presence of small amounts of adenylic acid in the blood plasma might conceivably be of importance under special circumstances as a secondary factor.

## SUMMARY

The presence of 2 to 5 mgm. per cent of 3-adenylic acid or of about 1 mgm. per cent of 5-adenylic acid in the blood plasma was accompanied by a distinct lowering of blood pressure in the dog. 5-Adenylic disappeared more rapidly than 3-adenylic acid from the blood stream.

It was possible to lessen the depressor effects of adenosine compounds by the injection of adenosine deaminase, adenylyl pyrophosphatase and alkaline phosphatase. These enzymes retained their activity in the circulating plasma for at least 1 hour after injection.

Although adenosine compounds could be demonstrated in the plasma coming from the injured limbs of shocked animals, the concentration in the plasma of the peripheral circulation was too low to account for the hypotension, and the injection of enzymes to inactivate possible adenosine compounds did not increase the blood pressure.

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# EFFECT OF FRONTAL LOBECTOMY ON BLOOD SUGARS OF NORMAL CATS AND MONKEYS AND ADRENAL DENERVATED CATS

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Removal of the cerebral cortex in cats is known to produce that syndrome of excessive activity of the autonomic system which has been termed sham rage by Cannon and Britton (4) and by Bard (2). Bilateral ablation of the frontal lobes of cats results in a similar condition of sham rage (7), probably because most of the cortical representation of the autonomic system lies in this part of the cerebral cortex.

Since many of the symptoms of sham rage, i.e., rise in blood sugar, in pulse and respiratory rates, and in blood pressure, can be effected by humoral agents, the possible interrelations of the endocrine glands with the central nervous system mechanism in question was obvious to the workers in Cannon's laboratory who were first engaged in the study of sham rage. And, since adrenalin production is known to occur in rage, the adrenals and their relation to the liberation of sugar were of particular importance. In this connection, Cannon and Britton (4) and Bulatao and Cannon (3) reported that the blood sugar of cats decorticated under ether anesthesia rose more than 100 per cent. Control experiments on animals under ether but without cortical ablation showed a high blood sugar level but no rise during the experimental interval. Such a procedure repeated in cats immediately after inactivation of the adrenals resulted in an initial rise in blood sugar, but this was unsustained, the sugar level falling rather rapidly at the end of the first hour after decortication.

The present investigation on the blood sugar changes in normal and adrenal denervated cats after ablation of the frontal cortex was undertaken for various reasons.

a. It was of interest to know whether frontal lobectomy produced all the changes which followed decortication, and to the same degree.

b. If the same changes in blood sugar level occurred after frontal lobectomy as after decortication, would they appear under another anesthetic such as barbiturate which did not cause, *per se*, a great rise in blood sugar?

c. Were such changes in blood sugar after frontal lobectomy altered by denervation of the adrenals?

d. Would similar changes in blood sugar appear in monkeys which never develop sham rage after cortical ablations?

METHOD. Thirty-six cats and five monkeys (*Macaca mulatta*) were used for this investigation. Ten experiments were discarded as will be noted later, the remaining 26 cats and 5 monkeys providing the basis for this report. The pro-

cedure was to anesthetize the animals, collect blood sugars at half-hour intervals thereafter (3 specimens) and then to perform the cerebral operation, continuing to collect blood every half-hour for 3 to 4 hours. Heart and respiratory rates were counted and recorded every 15 minutes. Blood pressure readings from a cannula inserted in the femoral artery were recorded in 3 cats and 3 monkeys. Healthy and well nourished animals were used. Experiments were begun about nine o'clock in the morning, the animals having been fed the usual laboratory diet on the preceding day.

*Anesthesia.* The barbiturates were chosen as anesthetics because of the even level of anesthetic they produce and because they have no effect on blood sugar. Dial (0.6 cc./kgm. intraperitoneally) was used in all monkeys and in 8 cats, and nembutal (0.5 cc./kgm. intraperitoneally) in the remaining cats. The two barbiturates were originally tried because there was some evidence that dial leaves a more excitable cortex than nembutal. But, as no difference in effect could be detected in our particular experiments, nembutal was used for many cats because of its shorter duration which was more desirable when the animals must survive for a second experimental procedure. Ether was given in 3 cases for comparison both with previous experiments of others and with our barbiturate experiments.

Careful attention was paid to the depth of anesthesia as it was essential to keep the levels as nearly alike as possible in all animals and to eliminate any change in blood sugar either from struggling on the one hand or from lack of oxygen circulation on the other. Three cat experiments were discarded because the anesthesia was so deep as to affect respiration and 5 because the level of anesthesia permitted movement and response to pain stimuli. Two further experiments were discarded because the cats were found to have respiratory infections after the procedure had started.

*Sugar determinations.* The method of Nelson (9) was used for blood sugar determinations. Two-tenths of one cubic centimeter of blood was collected from the femoral artery which had previously been exposed.

*Cortical operations.* Removal of portions of the cerebral cortex was made by suction. In the cats a burr hole was made in the bone above the desired cortical region and enlarged to necessary size. The dura was then excised and that portion of cerebral tissue desired was removed by gentle suction. In monkeys cerebral tissue was removed by blunt dissection and through a wide bilateral bone flap. Fifteen to 30 minutes was the usual time required for the entire procedure. In the cats, 15 bilateral frontal lobectomies were carried out. Five bilateral occipital lobectomies were made for comparison and 3 decortications. All above were under barbiturates. There were 3 additional frontal lobectomies under ether. There were 4 bilateral frontal lobectomies in the monkeys and 1 bilateral occipital lobectomy. In each instance all of the frontal cortex was ablated. Occasionally there was injury to the tip of the caudate nucleus as well. Occipital ablations were made with the object in mind of extirpating about as much tissue as was removed in the frontal ablations.

*Controls.* In 9 cats half hourly blood sugar samples under anesthetic were taken on one day without any operative procedure. A week or more later blood

sugar determinations were made on the same animals before and after cortical operations (5 frontal and 4 occipital lobectomies).

*Denervation of the adrenals.* The effect of denervation of the adrenals on blood sugar level after cerebral cortical ablation was tried on 6 cats. The right adrenal was removed and the left adrenal denervated some weeks before the rest of the experimental procedure. In the interval the appearance of hypoglycemic shock within 2 hours after the injection of 2 units of insulin per kgm. body weight was considered as evidence that the denervation was effective.

Anatomical verification of cortical lesions was made after removal and hardening of the brain in formalin. No histological sections were made.

**EXPERIMENTAL DATA.** *A. Cats.* The three frontal ablations under ether anesthesia resulted in confirmation of the facts already well known, that ether, *per se*, produces very high blood sugars. These experiments further confirmed the observations of Cannon and Britton (4) that cortical ablation causes still greater rise. Blood sugars were variable, however, and no further studies were made in this connection.

The effect on blood sugar of cortical ablations under barbiturate anesthesia can best be shown by the accompanying table and figures. In table 1 the blood sugar levels of all the 18 animals which had simultaneous bilateral cortical ablations have been summarized. It will be seen that these are *average* hourly blood sugars. For their compilation the average of either two, or three individual blood sugar determinations has been made for each hour. The difference in number of determinations per hour was due to the variable time which it took to perform the operations. An attempt was made to withdraw blood every half hour from the inception of anesthesia to the end of the experiment but when this schedule was altered by the operation, adjustments had to be made. Finally, when compiling the statistics, it was found that the *hour after operation* in which the blood was withdrawn was more significant than individual determinations (the latter can be seen in the figures). From the table, it will be seen that relatively very high sugars appeared in all but 2 instances, after the frontal operations in the cats, but that there was relatively little change following occipital operation.

In order to be sure that the blood sugar rise was not the result of anesthesia alone half hourly blood sugar determinations were made on 9 cats under barbiturate but without cerebral operation. A week later blood sugar samples were collected from the same animals both before and immediately after cortical ablation. A comparison of normal and postoperative blood sugar curves of 5 animals which had frontal lobectomies is shown in figure 1. Whereas there is no significant rise in blood sugar during 5 hours of barbiturate anesthesia, after bilateral frontal lobectomy there is a definite rise in 4 out of 5 cases beginning within the first half hour after operation and continuing at least 2 to 3 hours thereafter.

In figure 2 the effects of occipital lobectomy are shown in 4 cases. Although there is some rise in blood sugar level, it does not compare in degree with that which follows frontal lobectomy.

No relation between degree of blood sugar rise and size of lesion could be demonstrated in this series. Three total decortications (one of these in an adrenal-denervated cat) produced no greater changes in blood sugar than did

TABLE 1

*Hourly average blood sugar levels (mgm. per cent) before and after cortical ablations in cats under barbiturate anesthesia*

NO.	1ST HR.	OPERATION	2ND HR.	3RD HR.	4TH HR.
Frontal ablations					
38	69 100	fr.	83 128	90 174	184
39	77 127	fr.	100 137	97 230	91 275
43	64 102	fr.	59 104	59 98	58 90
41	64 117	fr.	62 161	63 257	57 247
62	104 97	fr.	94 130	87 172	110 166
45	90	fr.	100	128	132
69	130	fr.	152	151	147
70	75	fr.	79	82	99
Adrenal denervations					
33	85	fr.	116	115	108
34	71	fr.	109	103	120
54	123	fr.	156	191	174
55	128	fr.	167	181	153
56	112	fr.	172	167	145
57	141	occ.	136	132	110
Occipital ablations					
40	61 98	occ.	59 96	55 88	59 100
41	64 98	occ.	62 116	63 141	57 168
61	106 77	occ.	107 84	122 80	79
67	124 86	occ.	145 133	172 143	195 148

frontal lobectomy. Removal of a single frontal lobe in 2 instances and of one hemisphere in a third produced no changes but definite rise occurred in these animals after removal of equivalent tissue from the second side at a later date.

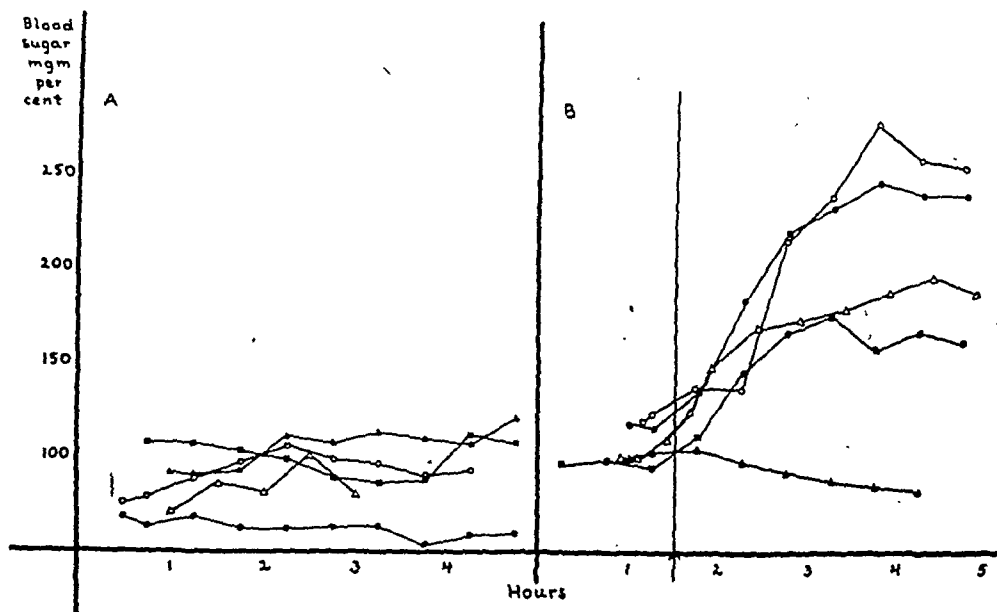


Fig. 1. Blood sugars of 5 cats under barbiturate anesthesia. A. During 5 hours without operation. B. After bilateral frontal ablation. Arrow signifies time of operation. More than one week elapsed between times of A and B.

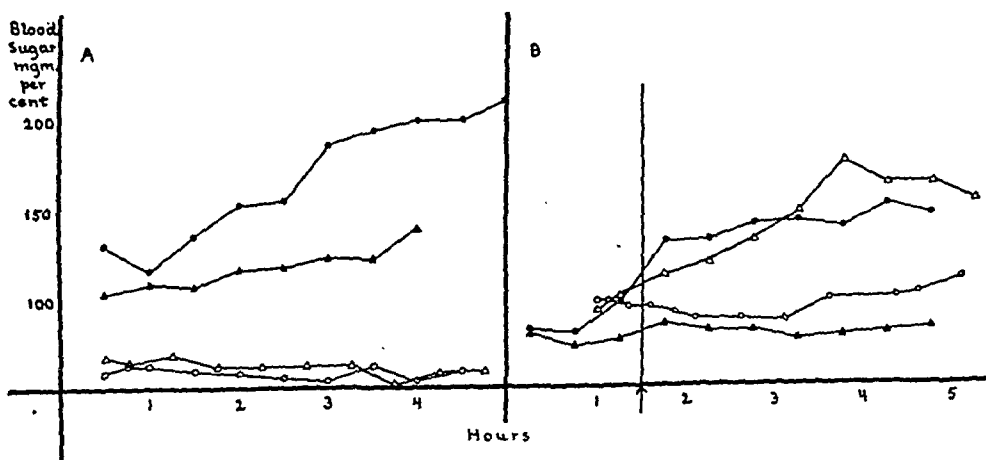


Fig. 2. Blood sugars of 4 cats under barbiturate anesthesia. A. Without operation. B. After bilateral occipital ablation. Arrow signifies time of operation. More than one week elapsed between A and B.

The adrenals of 6 cats were denervated as described above. Figure 3 shows the blood sugar curves before and after cortical ablation in these chronically adrenal-denervated animals. When compared to figure 2 it can be seen that the

blood sugar of the adrenal-denervated animal rises as promptly after frontal ablation as does the sugar of the animal with intact adrenals, but that there is, within 3 hours after operation, a fall in the level of blood sugar in the adrenal-denervated animals (fig. 3) whereas the level remains high when the adrenals are intact (fig. 2).

*Pulse and respiration.* An increase in heart and respiratory rates, although less regular and less marked, generally accompanied the rise in blood sugar. In the unoperated control group the pulse and respiration usually settled within 15 minutes after anesthetic had become fully effective to a level which was maintained fairly constant throughout the experimental period. In a few instances

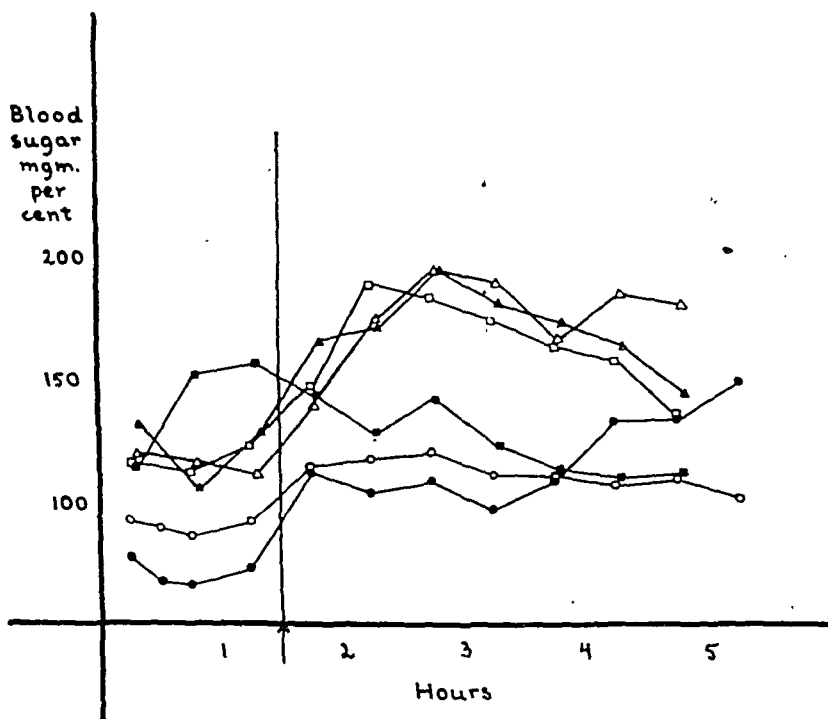


Fig. 3. Blood sugars of 6 cats under barbiturate anesthesia before and after frontal lobectomy. The adrenals of these animals had been previously denervated.

both pulse and respiration increased slightly toward the end of the 5 hour period at which time blood sugar also increased. But after either occipital or frontal operation an increase in pulse and respiration accompanied a rise in blood sugar of any degree. This was true in the adrenal-denervated cats as well as in the normal animals.

*Blood pressure.* In contrast to pulse and respiratory changes, mean blood pressure levels determined by direct cannulation of the femoral artery in 3 cases were unchanged throughout the duration of the experiments.

*B. Monkeys.* Figure 4 shows the blood sugar levels of 5 monkeys (*Macaca mulatta*) before and after bilateral frontal ablations (4 cases) and occipital abla-

tion (1 case). In contrast to the changes which occurred in the cats, no significant rise in blood sugar appeared in any instance in the monkeys. In all cases, pulse, respiration and blood pressure remained equally unchanged by these cortical excisions.

**DISCUSSION.** The most interesting point in this investigation is that the blood sugar, pulse and respiration of cats all rise immediately after frontal lobectomies at a time when the animals are so deeply under anesthetic that they are relaxed and motionless. All evidence points to the fact that these changes are part of the mechanism which, in the unanesthetized animal, produces the full picture of rage for:

1. These manifestations appear only after the operative procedure which causes sham rage—namely, bilateral frontal lobectomy.

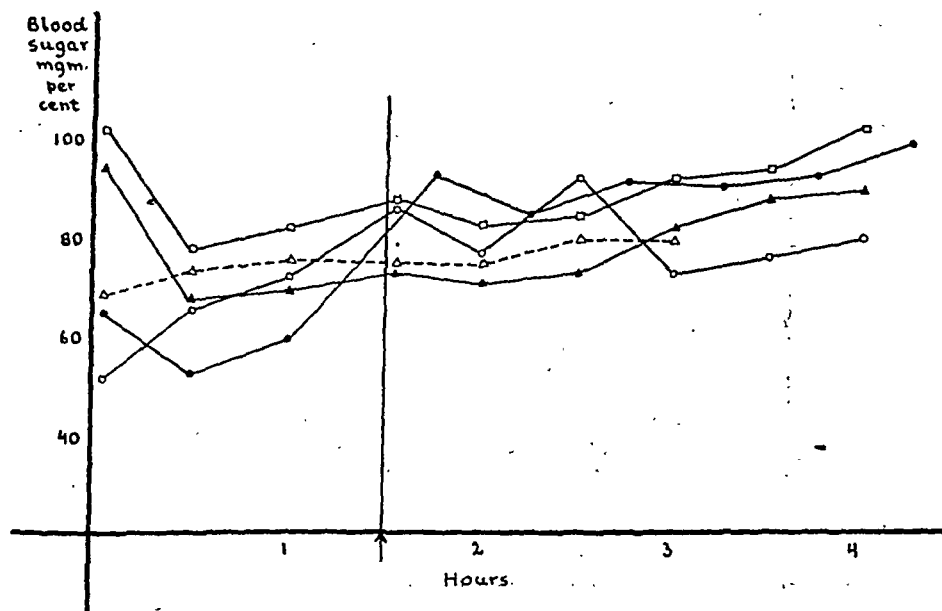


Fig. 4. Blood sugar of monkeys (*Macaca mulatta*) before and after bilateral frontal (4 cases) and occipital (1 case, broken line) lobectomies.

2. It appears only in that animal in which sham rage can be produced, i.e., the cat—not in the monkey which, although it has cortical representation of the autonomic system limited chiefly to the frontal lobes, never shows any trace of rage manifestations after severance of these regions from the subcortical areas.

Even the fact that in 2 instances (figs. 1 and 3) no rise in blood sugar followed frontal ablation may be made to fit into this picture. No cause was found for these deviations—either physiological or anatomical, but long experience with acute decorticate cats has taught that there is an occasional animal which, on recovery from anesthetic and after an apparently optimal operation, shows no signs characteristic of sham rage. It is possible that in subsequent experiments wherein we intend to make careful fractional cortical ablations, some focal cause for these differences may be observed, within the central nervous system.

The observation that there is no accompanying rise in blood pressure when pulse and blood sugar levels change is of interest in relation to the findings of Leimdorfer, Arana and Hack (8) that intracisternal injection of adrenalin into cats produces a marked rise in blood sugar but no change in blood pressure. Intraperitoneal injection of adrenalin, on the other hand, causes a marked rise in both.

Our data are of further interest in relation to the problem of sham rage. It has been clear that, with encephalization, the more primitive reactions of the autonomic systems are no longer so markedly influenced in animals with more complex forebrains such as monkeys as they are in the animals with simpler forebrains such as cats (7). The relation of the reaction of each animal to sympathetic or to parasympathetic dominance has also been discussed. Our observations show, as contributory to this problem, that cortical ablations which in the monkey result in hyperactivity and in the cat in sham rage, have different basic autonomic patterns.

#### SUMMARY

A. In cats in which sham rage can be produced by bilateral frontal lobectomy:

1. The blood sugar level under dial or nembutal anesthesia is increased within one-half hour following frontal lobectomy, and may continue raised for at least 4 to 5 hours thereafter.

2. No such rise in blood sugar occurs in cats which are anesthetized with barbiturates without frontal lobectomy.

3. Bilateral removal of an amount of occipital lobe approximately equal to that of bilateral frontal lobectomy causes no such rise in blood sugar.

4. There is an accompanying rise of pulse and respiratory rate when blood sugar rises.

5. Previous adrenal denervation causes the blood sugar rise which appears after frontal lobectomy to fall within 3 to 4 hours.

6. No rise in blood pressure occurs after frontal lobectomy although blood sugar, pulse and respiration increase.

B. In monkeys, which do not develop sham rage after cortical ablations, there is no significant change in blood sugar, pulse, respiration or blood pressure after bilateral frontal or occipital lobe ablation.

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# THE RELATION OF LOAD TEST RESPONSE AND FASTING EXCRETION LEVELS TO TISSUE CONTENT OF THIAMINE AND RIBOFLAVIN

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Biochemical data are frequently used in the appraisal of nutritional state, and where vitamins are concerned, such data may be of three types: a, the fasting urinary excretion level; b, the load test response; c, the blood level. Of these, the first named is simplest to obtain, therefore it is used most. Its significance is questioned by some, however, chiefly because the level of intake of a vitamin for the immediately preceding two or three days appears to be the factor most important in determining the level at which that vitamin is excreted in the urine (1, 2). The investigator may be misled, therefore, in cases where a temporarily low level of vitamin intake is reflected in a low urinary excretion level, even though the actual state of nutrition may be excellent. The converse is met in cases of transiently high intakes. In consequence, the possibility arises that load tests, although more tedious, may reflect more accurately the concentration of vitamins in the tissues, and therefore, actual nutriture. Melnick and co-workers (3) have suggested the use of a test dose, given with a meal, as a means of indicating the adequacy of thiamine stores in body tissue, and they derive an "index of thiamine nutrition", based on the amount of the test dose which is excreted in the urine. Gyorgy (4) states that in a large number of investigations, the saturation test, with various but not essential modifications, has been found to be the reliable way to assess a possible deficiency of thiamine. The work of other investigators would appear to confirm such a conclusion (5, 6, 7, 8), although it would seem likely that factors other than the nutritive state of the tissues could affect load test response; among these are rate of absorption of the test dose, the renal threshold level, and the length of the collection period. If the more ideal method of administering the test dose by intravenous or intramuscular injection is used, the simplicity required for field surveys is lost because of the added requirements of aseptic technique.

The present report deals with laboratory experiments designed to obtain more information on the relative merits of load tests and fasting excretion levels of vitamins. The general plan of the experimental work had two aspects: 1, to feed to each of several groups of adult rats a level of thiamine that would result in the depletion of that vitamin from the tissues, and to correlate the resulting urinary and load excretions with the rate of decrease of thiamine concentration in liver, kidney and heart, and 2, similarly for riboflavin, using separate groups of animals. In general, a group of four rats was taken at each of the progressive stages of depletion, and after fasting excretion levels were determined, two of

the animals were used on the following day for the determination of load test response, and the remaining two were sacrificed for tissue analyses. This group of determinations was made weekly for 20 weeks in the case of thiamine. In a second shorter experiment, the determinations were done on five successive occasions four days apart. In the case of riboflavin, two initial experiments were run, with the analytical determinations being carried out once weekly for periods of eight weeks. A third experiment was then carried out over a seven week period, when the analyses were done twice a week.

*Animals.* Normal male albino rats weighing approximately 250 grams were used. These animals were maintained in groups of four and were permitted access to the basal ration and water *ad libitum*. Care was taken to avoid mixing different strains. Control animals receiving an adequate diet were used throughout as a check on the results obtained.

*Diet.* The diet used for both types of experiments (i.e., thiamine and riboflavin depletion) was the same, being essentially free of vitamins, and having the following composition:

	Per cent
Vitamin test casein (SMACO).....	20
Dextrose.....	68
Corn oil.....	8
Salt mixture (Kline et al. (7)).....	4

The following supplements were added to this basal diet:

	mgm. per 100 grams diet
Choline.....	400
Nicotinic acid.....	10
Pyridoxine.....	1.2
Ca pantothenate.....	5.0
Thiamine (for animals depleted in riboflavin only).....	1.0
Riboflavin (for animals depleted in thiamine only).....	1.6

In addition, each animal received weekly 2 drops of corn oil containing 200 I.U. vitamin A, 2 I.U. vitamin D from haliver oil, and 2 mgm. alpha tocopherol. Levels of thiamine or riboflavin intake were varied for the different groups of animals by merely changing the quantity of the thiamine or riboflavin supplement.

*Sample collection and analysis.* Analyses for fasting excretions, test dose response, and tissue content were conducted in the following manner: four animals comprising the group to be used at any one stage of depletion, were placed in individual metabolism cages late in the afternoon, with access to water but not food, and urine was collected continuously for a 16-hour period. The urine was allowed to fall directly into an oxalic acid solution (thiamine), or acetic acid (riboflavin), used as preservatives. At the end of that period, two of the animals were killed and the desired tissues removed and placed immediately in a deep-freeze unit until prepared for analysis. Shortly thereafter, the load test was begun on the remaining two animals which received by tube a load test dose of 200 mcg. thiamine, or 400 mcg. riboflavin. They were then returned

to metabolism cages for another period of 16 hours, this collection period being considered adequate for measuring load test excretion.

Urine was assayed for thiamine, following adjustment to pH 4.5, by the method of Conner and Straub (9). Riboflavin in urine was determined by a modification of the method of Najjar (10). (The modification consisted of measuring the fluorescence of riboflavin in aqueous medium, turbidity being excluded by the use of small samples of material. In each set of determinations, internal standards were run separately by adding known quantities of pure riboflavin to extra duplicate samples of the material analyzed.) These same analytical methods were used for the analyses of thiamine or riboflavin in tissues after preliminary preparation as follows: liver, kidney and heart were prepared by grinding with 0.1 N  $\text{H}_2\text{SO}_4$  in an all-glass homogenizer, then heating at  $70^\circ\text{C}$ . for a period of 1 hour. After cooling, the samples were made up to volume, and suitable aliquots were combined with one-fifth volume of 6 per cent takadiastase in 2.5 M sodium acetate buffer, and then incubated overnight at  $37^\circ\text{C}$ ., as described by Mitchell and Isbell (11).

**RESULTS.** *Thiamine.* When intake of thiamine was shifted from the original control intake of 1000 mcg. (per 100 grams of moist diet) to an experimental intake of 1 mcg., there was a prompt and immediate drop in both fasting and load excretion levels by the end of ten days, and a further drop was found to have occurred by the end of 20 days. These changes were accompanied by similar decreases in the amounts of thiamine found in liver, heart and kidney. These marked effects were deemed to be similar to those found in severe deficiency, where it is known that both load and fasting excretion levels are markedly depressed. Since we desired to determine the effects during a slower rate of depletion, measures were then taken to increase the amounts of thiamine given to the remaining animals. For the next 15 days intake was at the 5 mcg. level, for the next 50 days at 25 mcg., and for the next 15 days at 50 mcg. For the final 40 days of the experiment, the intake was dropped to a 15 mcg. level.

The changes in excretion levels following these increased intake levels are shown in figure 1. It will be observed that there was but little difference between load test and fasting excretion until the 50 mcg. intake level was reached. At that point, however, there was a marked increase in load test response which far exceeded that found to occur at the lower supplementation levels. It will be noted that the tissue content of thiamine was also greatly increased at the same time. It is apparent that during this process of repletion of the tissues, fasting urinary excretion did not show any great increase, while load test response, on the other hand, did increase markedly. This would appear to be good additional evidence that the fasting excretion level is dependent primarily upon current or immediately preceding dietary "excess" (this term denoting those amounts of a vitamin which are absorbed but not taken up by the tissues). It will be noted also from figure 1 that when the intake of thiamine was subsequently decreased to the 15 mcg. level, the change in fasting excretion was rather slight in comparison with the definite drop in load test response and tissue content. This latter finding points up one advantage of load test response over

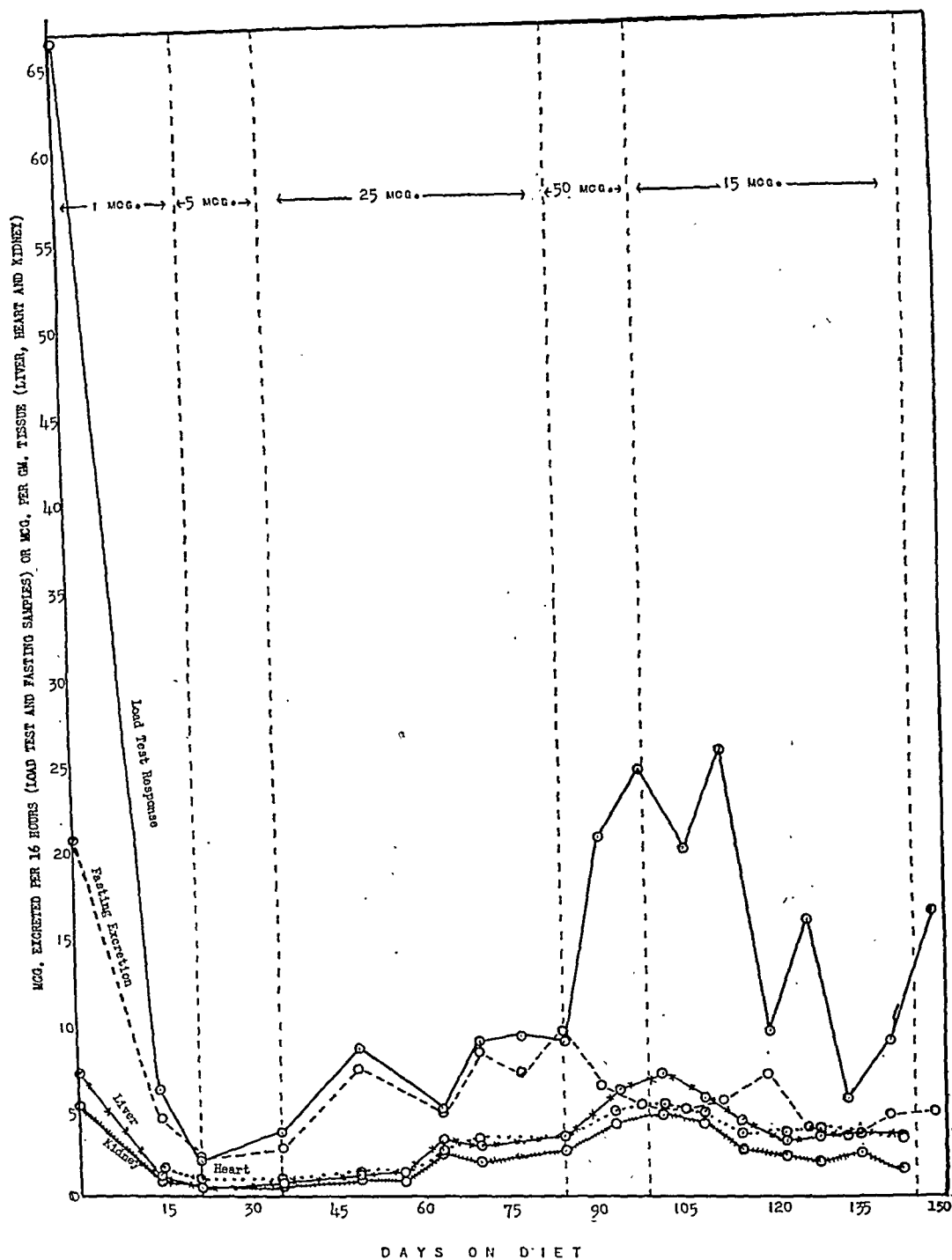


Fig. 1. Thiamine load test response, fasting excretion levels and content in liver, heart and kidney at various intake levels.

fasting excretion, namely, the magnitude of the former is usually sufficient to permit the analytical detection of decreases somewhat more easily during the

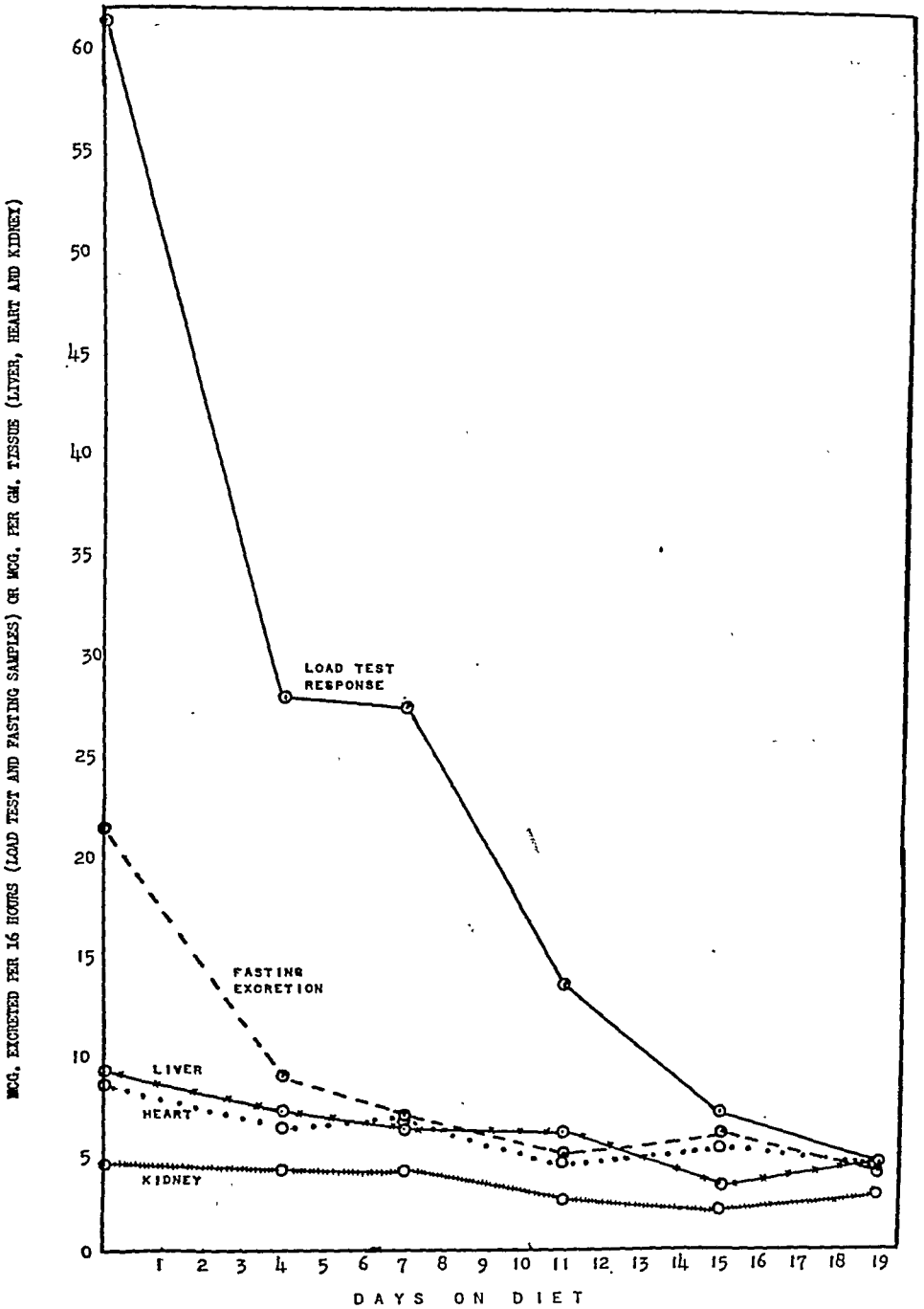


Fig. 2. Thiamine load test response, fasting excretion levels and content in liver, heart and kidney at intake levels of 16.6 mcg. per day.

course of depletion, and for a longer period of time, than is the case with fasting urinary levels.

The foregoing experiment not only provided data showing trends at different levels of thiamine intake, but also gave an indication of the level of thiamine

intake which would be likely to cause a slow and steady depletion. In a subsequent experiment, the same techniques were used, but the level of intake was maintained throughout at 100 mcg. per 100 grams of moist diet. The findings in this experiment are shown in figure 2. It was found again that the rate of decrease (liver, heart, kidney) in tissue levels was more accurately reflected by the changes in load test response rather than by fasting urinary excretion. The latter dropped initially within four days after the beginning of the 100 mcg. dietary regime, and thereafter remained at low static levels for the duration of the experiment. This precipitous change was far different from the gradual and continuing decrease that was found to be occurring in the tissues. The decrease

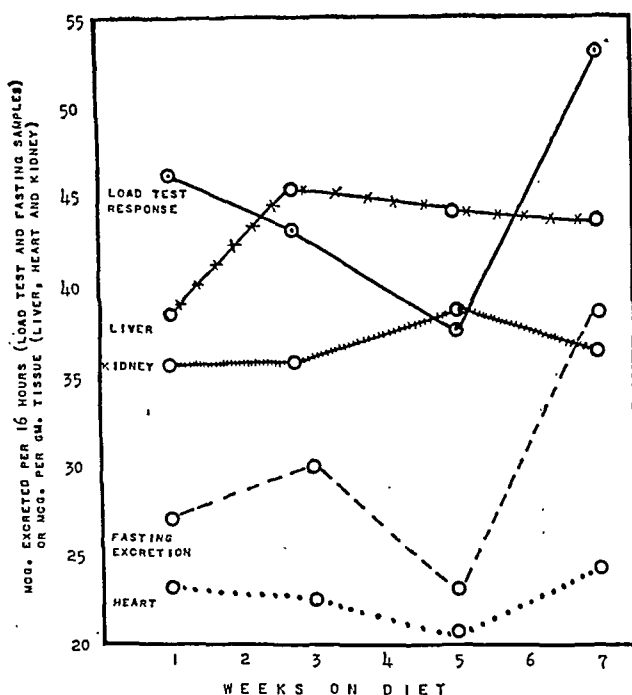


Fig. 3. Riboflavin load test response, fasting excretion levels and content in liver, heart and kidney at intake levels of 265 mcg. per day (controls).

in load test response, on the other hand, was more gradual and quite similar to that occurring in the liver, and to a lesser extent in the heart and kidney.

*Riboflavin.* Four levels of riboflavin supplementation were used as follows: 1600 (controls), 240, 120 and 60 mcg., respectively per 100 grams of diet. Since the intake of food per rat per day was approximately 16.6 grams, the riboflavin intake for each of the four groups was 265, 40, 20 and 10 mcg., respectively. Figures 3, 4, 5 and 6 depict the changes on all four levels. On the control level it was found that there was no consistent decrease in either fasting or load test excretion. The tissue content of riboflavin was also maintained. On the 40 mcg. level, both fasting and load excretions dropped, but the changes in liver, kidney and heart were not marked. At both the 20 and 10 mcg. levels, however, it became quite apparent that the rate of decrease in liver content of riboflavin

was similar to the rate of decrease in load test response, whereas fasting excretion had dropped promptly following the low intake regime, was more or less fixed thereafter, and could be represented by a flat line on the graph.

These results are similar to those obtained for thiamine in that they show clearly the relationship of load test response to the level of a vitamin present in the tissues, particularly the liver. It is quite obvious from figures 5 and 6 that when there is no dietary "excess" (*vide supra*), fasting excretion levels are continuously low. Load test response, on the other hand, declines gradually

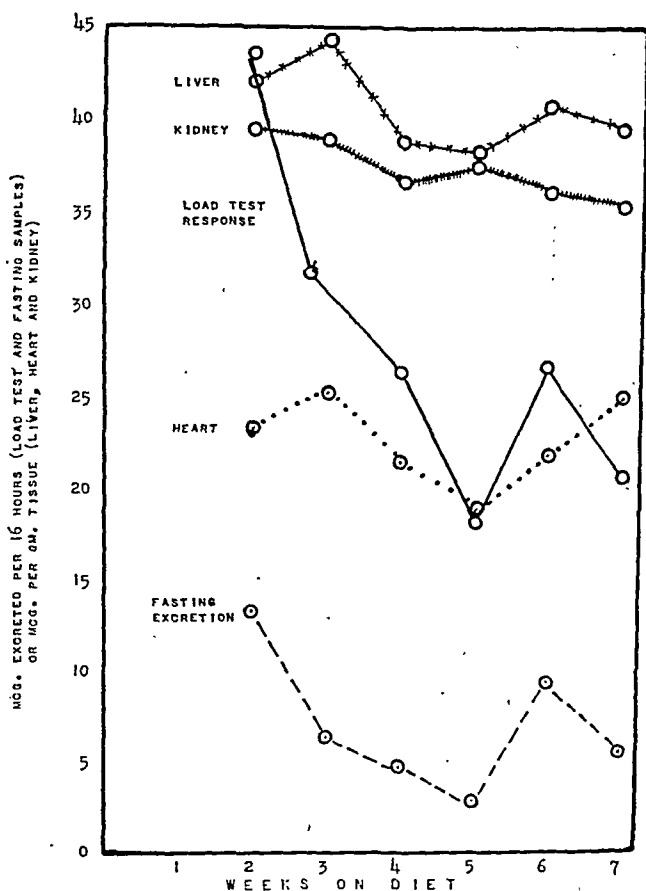


Fig. 4. Riboflavin load test response, fasting excretion levels and content in liver, heart and kidney at intake levels of 40 mcg. per day.

(rate of decline being dependent on the level of intake of the nutrient), and roughly in proportion to the rate of decline in the riboflavin content of liver, kidney and heart.

It appears that the riboflavin content of liver is affected before that of kidney or heart. Table 1 shows the relative rates of depletion on different levels of intake of riboflavin.

DISCUSSION. The results of these experiments on both thiamine and riboflavin indicate that decreased dietary intake results in an immediate drop in

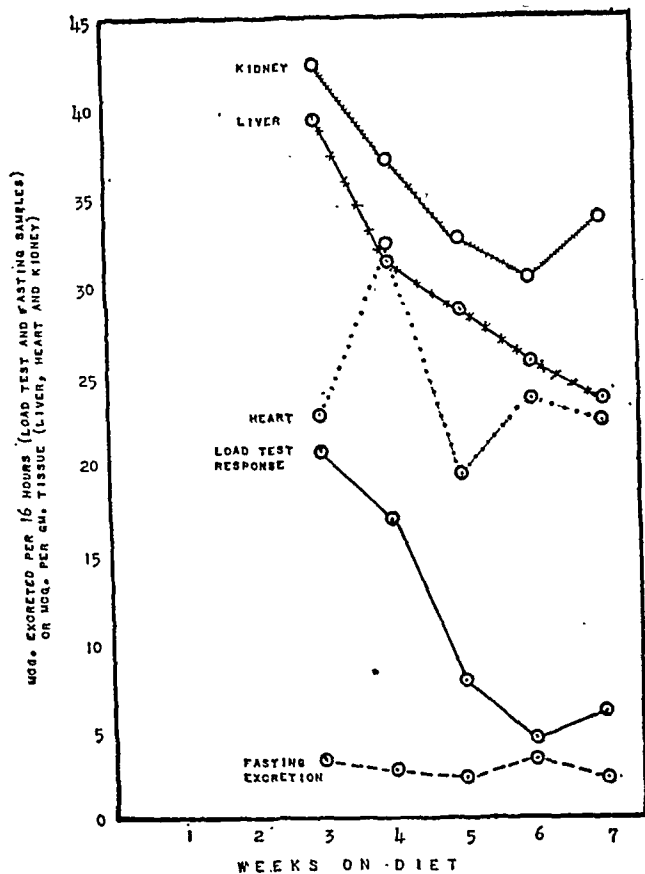


Fig. 5. Riboflavin load test response, fasting excretion levels and content in liver, heart and kidney at intake levels of 20 mcg. per day.

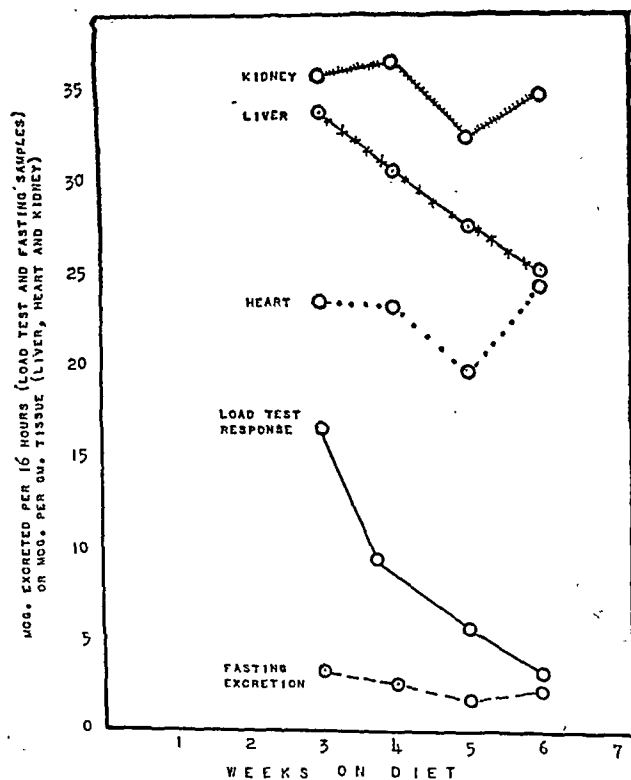


Fig. 6. Riboflavin load test response, fasting excretion levels and content in liver, heart and kidney at intake levels of 10 mcg. per day.

the fasting urinary level. That a decrease in true nutritional state is an inevitable consequence does not necessarily follow, for it could be possible from the theoretical standpoint to so delicately balance the intake of any nutrient against metabolic requirements that no excess would appear in the urine, even though the nutritional state of the internal milieu were nicely maintained. In actual practice, however, this probably happens only rarely or momentarily, and the

TABLE 1  
*Rate of riboflavin depletion from liver kidney and heart\**  
(mcg. per gram tissue)

WEEK	TISSUE	CONTROL	AT INTAKE LEVEL OF 40 MCG. PER 100 GRAMS DIET	AT INTAKE LEVEL OF 20 MCG. PER 100 GRAMS DIET	AT INTAKE LEVEL OF 10 MCG. PER 100 GRAMS DIET
1	Liver	38.55			
	Kidney	35.72			
	Heart	23.29			
2	Liver		41.98		
	Kidney		39.28		
	Heart		23.18		
3	Liver	45.49	44.06	39.26	33.90
	Kidney	35.83	38.89	42.25	35.95
	Heart	22.43	25.18	22.76	23.58
4	Liver		38.69	31.39	30.63
	Kidney		36.49	36.96	36.51
	Heart		21.38	23.26	23.39
5	Liver	44.27	38.08	28.45	27.59
	Kidney	38.91	37.25	32.66	32.59
	Heart	20.76	18.74	19.27	19.90
6	Liver		40.83	25.65	25.24
	Kidney		35.94	30.25	34.85
	Heart		21.84	23.53	24.33
7	Liver	43.83	39.57	23.67	
	Kidney	36.41	35.26	33.67	
	Heart	24.12	24.78	22.16	

\* Each figure represents average of 2 animals.

likelihood is that continued low fasting excretion levels would be followed ultimately by a decreased tissue content, and this in turn followed by more gross signs of nutritional lack. On the basis of the present findings in rats, it is conceivable that in the development of a gross deficiency state in the human, the following sequence of events would take place: 1, decreased dietary intake or increased metabolic requirements; 2, reflection of this change by a relatively

rapid decrease in the urinary excretion levels; 3, a less rapid decrease in load test response caused by 4, a gradual but definite decrease in the vitamin content of the liver and other tissues, followed by 5, ultimate microscopic and macroscopic pathology resulting in impaired physical and psychomotor performance, and the manifestation of clinical deficiency signs and symptoms.

#### SUMMARY

In adult rats receiving less than adequate intakes of thiamine, and in others receiving less than adequate intakes of riboflavin, but adequate quantities of other nutrients in both cases, it was found that the rate of decrease in the thiamine or riboflavin content of liver, heart and kidney was reflected correctly by corresponding changes in load test response, but not by changes in fasting urinary levels which appeared to be more closely governed by the *immediately preceding* level of thiamine or riboflavin intake. This would indicate that load test response is more significant than fasting urinary levels in the appraisal of nutritional state. Thiamine and riboflavin levels in fasting and load test urine samples, and the thiamine and riboflavin content of liver, heart and kidney are reported for different levels of intake of those vitamins in the rat.

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# ELECTROCARDIOGRAPHIC CHANGES INDUCED BY COOLING OR WARMING THE INNER SURFACE OF THE DOG'S VENTRICLE

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One of the important questions with respect to the genesis of the electrocardiogram concerns the contribution made by the depolarization and repolarization of the inner or endocardial lamina of muscle fibers. This problem has been approached in several ways. Lewis studied premature beats induced by stimulation of the inner (endocardial) or outer (pericardial) surface of the right ventricle, the electrocardiographic derivations being from the right chest wall to the left chest wall. The lead poles were so arranged that relative negativity of the right chest wall yielded an upward deflection, and vice versa. Lewis found that when the outer surface was stimulated, the QRS complex was initiated by an upward (negative) deflection, whereas, when the inner surface was stimulated the initial deflection was downward (positive) (1). This experiment appeared to demonstrate that depolarization of the endocardial surface of the ventricle led to the appearance of significant potential differences between the two sides of the thorax. More recently Nahum and Hoff reported a series of experiments in which Lewis' observations were repeated and extended (2). Their interpretations differ from those of Lewis. They found that both in transthoracic and in "unipolar" leads, when the one lead pole was directly over the stimulated region of the heart wall, the form of the QRS complex of the forced beat was the same, whether the inner or outer surface was stimulated. From these, and from further experiments, they conclude that, at least in the limb leads, activity of the endocardial muscle layers before the wave of excitation has broken through to the epicardial surface contributes little or nothing to the deflections of the electrocardiogram. They also extended their conception to include the T wave. Their conclusions are clearly stated in the 15th edition of Howell's *Text-Book of physiology*, p. 758.

Also relevant to the question are reports on the electrocardiographic effect of injuring the subendocardial muscle lamina of the left ventricle (3, 4, 5). For the most part the effect on the RS-T segment in the limb leads has been slight, negligible or absent, and fits into no consistent pattern of electrical change. Wolferth et al., however, found that in direct leads from the epicardial surface of the heart, injury of the underlying endocardial surface produced downward displacement (negativity) of the RS-T segment (5). In one case, a slight depression of RS-T was observed in Lead II. In this connection a recent report of Bayley is of particular interest (6). Several electrocardiograms had been taken on a patient before death. These showed conspicuous depression of the RS-T segments, especially in Leads I and II, and in the precordial lead. At autopsy and upon histologic study of the heart it was found that the major damage involved the

endocardial muscle layers. The displacement of the RS-T segments was in the direction which, in theory, should have resulted from preponderant endocardial injury.

The technical difficulties and uncertainties attending experiments designed to solve the problem of endocardial contribution to the electrocardiogram are formidable. The methods used to produce injury not only destroy the ventricular muscle proper; usually in a haphazard and irregular fashion, but they will also destroy subendocardial Purkinje fibers and change, in an unknown manner, the order of activation of the muscle superficial to the injury. The results are, consequently, not necessarily comparable with those produced by disease, in which the Purkinje fibers will often be less damaged than the ventricular muscle proper. Another difficulty, both in the induction of injury and in electrical stimulation, is that the thorax must be opened, and in replacing the tissues surrounding the heart, the normal relationships are not necessarily restored, and air pockets may sometimes overlie the surface in which the larger electrical disturbances appear. Often, also, injury of a significantly large area of the subepicardial muscle will inadvertently be produced. A third difficulty in injury experiments relates to time. The current of injury of a cut surface, bathed by blood, may possibly fall off in intensity more rapidly than the injury current from an injured surface which is not directly washed by the blood because of the presence of the intact connective tissue endocardium (5).

Our method of changing the temperature of the subendocardial surface relative to the epicardial seemed to offer a way out of these difficulties. Mammalian Ringer's solution at various temperatures can be introduced into the ventricular cavity by catheter with little or no injury of the heart and without disturbing the contact of the heart surface with the surrounding tissues. Furthermore, the electrocardiogram is recorded at the time the change in temperature is present.

The information sought in these experiments was whether or not a change in the physiological response of the subendocardial muscle lamina influences the form of the electrocardiogram, particularly the T wave. It is almost certain that if changes occur during repolarization of the muscle, the same fundamental principles will also apply to the electrical changes associated with depolarization, and also to the electrical effects of injury.

**METHOD.** Large dogs under nembutal anesthesia were used. The left common carotid artery (or jugular vein) and the right vagus nerve were exposed in the neck. Arrangement was made to faradize the vagus. A catheter was introduced into the left ventricle by way of the carotid, or into the right ventricle by way of the external jugular vein. In each experiment Lead I, II, or III, or the precordial (CF) lead, was being taken while one of the following procedures was followed: (a) Faradization of the right vagus, resulting in cardiac standstill (barring escapes in some experiments) for several seconds. (b) Faradization of the vagus together with the injection of 50 cc. of Ringer's solution through the catheter from a syringe, the injection being completed just before cessation of vagus stimulation. (c) Repetition of (a) in nearly all cases. In each case a few control beats were recorded before and many beats were recorded after vagus

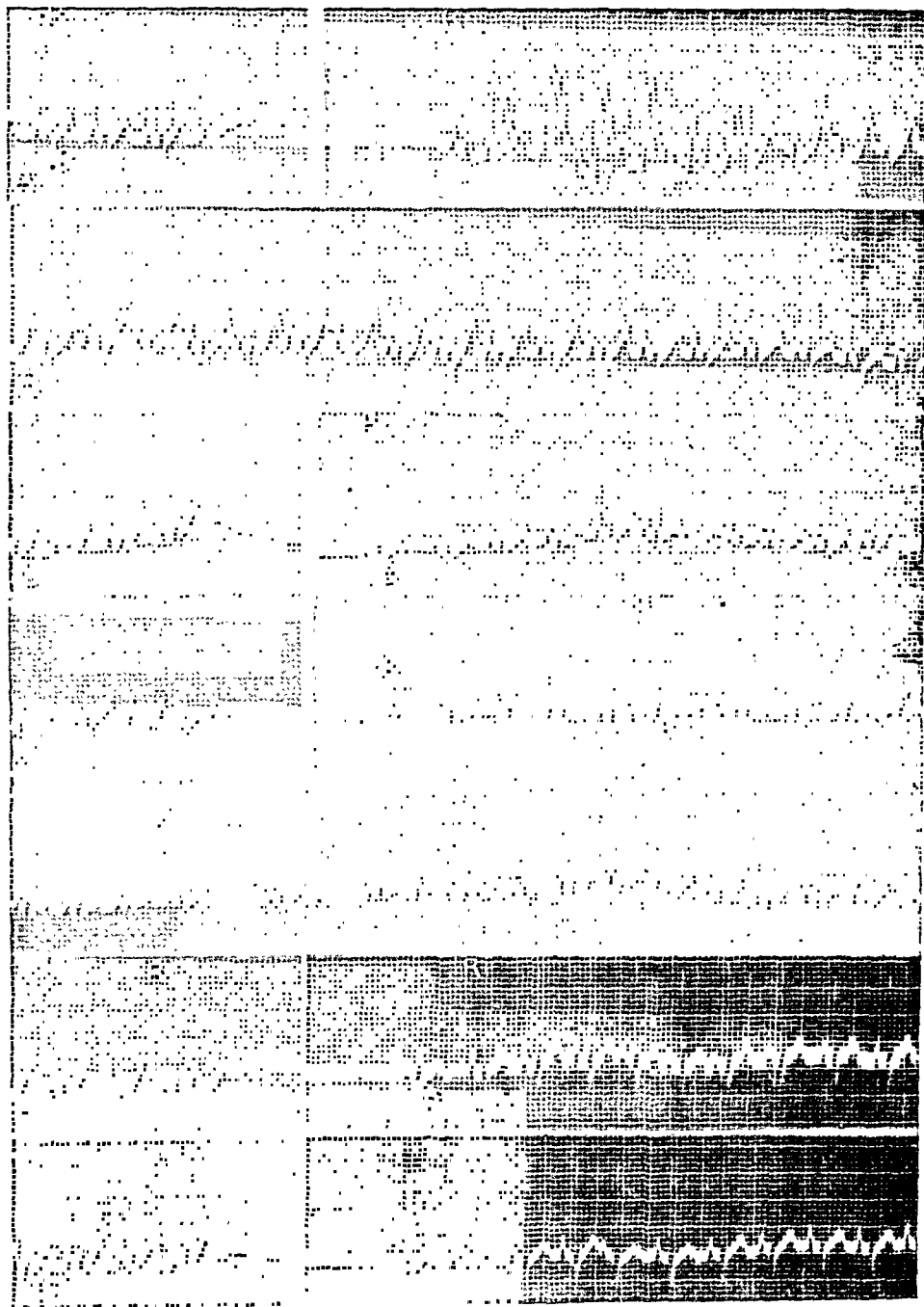


Fig. 1. Soft rubber catheter, three openings, within apex of left ventricle. All Lead II, from same dog.

Line A: Vagal arrest during which time 50 cc. of Ringer's solution at  $1^{\circ}\text{C}$ . were injected; 6.0 sec. omitted, with two escaped beats. Note the large increase in area, both of the R and T waves.

Line B: Continuous with A. For a short time following B (not shown), the T waves became lower than in the control.

stimulation. The stimulation was necessary to permit the injected solution to change the temperature of the inner ventricular wall before being forced out by the contractions. The temperatures of the solutions were noted as they entered the syringe, but they had no doubt often risen or fallen by the time they reached the ventricular cavity. In half the experiments, heparin was used to prevent clotting.

At the end of each series of experiments, the dog was killed and the location within the ventricle of the tip of the catheter was observed.

**RESULTS.** *Cooling the Inner Surface of the Left Ventricular Apex.* *T-wave changes.* In 24 experiments on 8 dogs, the tip of the catheter was definitely located within the left ventricle near the apex. The Ringer's solution went into the syringe at temperatures ranging from 10° to 0°C., but usually 1° to 3°C. In all these experiments the T wave in the CF<sub>4</sub> precordial lead (10 expts.) and in Lead II (12 expts.) was definitely, and usually conspicuously, increased in height and width (fig. 1). In two dogs, in Lead II, in association with great increase in the area of the R wave, the T waves during cooling were diphasic, the second phase being upright and the Q-T was prolonged. The net QRS-T area was greatly increased. In Lead I (2 expts.) the T was increased in one, rendered diphasic in the other, but with very slight increase in net QRS-T area. In the same dog, T<sub>2</sub> was greatly increased. The findings demonstrate, as was expected, that the electromotive force representing the effect of cooling is oriented nearly parallel to the long axis of the dog's body, but is directed slightly to the left as well as caudad. This is a consequence of the vertical position of the dog's heart.

In two experiments on one dog, an ECG labelled 39° showed a change like that due to cooling, and another curve labelled 1°C. was like the controls. We have no doubt that there was an error in labelling in these, but they are not included in the number of the other experiments which revealed consistent findings.

In 6 experiments (Lead II) Ringer's solution at 39° or 40°C. produced no appreciable changes in the T waves; these waves were the same as the waves following the period of vagus arrest of the heart. In one experiment, Ringer's at 39°C. seemingly produced a very inconspicuous increase in the T wave, whereas the cold solution in the same dog produced a very great increase. In no other trial (with the probable error noted in the last paragraph) did the solution near body temperature produce a measurable T-wave change.

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Line C: Record taken about a minute after B. Vagal arrest; no injection; 6.8 sec. omitted; no escaped beats. Note effect of slowing on T waves. Another control, a few minutes before A, was like C.

Line D: Vagal arrest, with injection of 50 cc. of Ringer's solution at 65°C.; 5.4 sec. omitted, with six escaped beats. Note inversion of T in spite of reduced QRS area of first beat after warming.

Line E: Continuous with line D. Controls, with vagal arrest, both before and after this experiment, were like line C.

Line F: Vagal arrest, with injection of 50 cc. of Ringer's solution at 40°C.; 5.0 sec. omitted, with two escapes.

Line G: Taken shortly after F. Vagal arrest, with no injection; 6.8 sec. omitted, with one escape. Other controls were like this one or line C.

For the majority of these 31 experiments (24 with cooling and 7 controls), another control was done, namely, vagus stimulation without injection of Ringer's solution, both before and after the effect of the injection had been recorded. In connection with all experiments, shortly before or after, at least one vagus control of this sort was recorded. The average duration of cardiac arrest was the same in trials with and without injection, except in those dogs in which ectopic beats induced by the injection shortened the period of arrest. As a rule, the T waves following the arrest were slightly larger than before, and in one dog this effect was rather conspicuous. In other experiments, without change in the QRS complexes, the T waves became more deeply notched or slightly inverted. The effects of temperature change were, of course, estimated in reference to the effects due simply to arrest.

*Q-T interval changes.* The Q-T interval was consistently lengthened by cooling. The increase with the colder solutions ranged from about 60 per cent to over 90 per cent. The beats after arrest alone, or after injection of Ringer's at 39° or 40°, usually showed an increase in Q-T of 5 to 10 per cent.

*QRS-complex changes.* The QRS complexes were widened, sometimes even doubled, in duration. In the precordial lead, the changes in amplitude of the R and S waves were not so great as in Lead II, nor were they so consistent. In Lead II, the R waves were always widened; they increased in amplitude slightly or even by over 50 per cent, whereas the S waves, if present, were greatly reduced in amplitude or practically eliminated.

*Persistence of effects.* After the end of injection, as the rate returned to the original one, the T waves grew progressively smaller. In a few hearts, alternation in T-wave amplitude was observed during this period. Full return to the control T-wave height required between 10 and 15 seconds. In some dogs, the T waves, low but upright in the control, were greatly increased by the cool solution and then, gradually decreasing in height, became inverted 12 to 15 seconds after the injection. Within a minute or two they had reverted to the control form (fig. 1).

The QRS complexes returned to the control form within 4 or 5 seconds, i.e., more quickly than the T waves. It may be noted that, because of the quickness of movement of the galvanometer string during inscription of these deflections, slight changes from the control in their shape and duration could not be easily recognized.

*Other effects of cooling.* Fairly consistently, the first beat after the arrest revealed less increase in T-wave height than the second, third, or fourth. The meaning of this is not clear.

*Cooling the Inner Surface of the Left Ventricular Base.* In three dogs, autopsy revealed quite definitely that the tip of the catheter was caught behind the mitral valve or chordae tendineae in such a way that the injected solution must have entered the ventricle behind the valve leaflets at the base of the ventricle. The electrical changes differed strikingly from those described above. 1. The change in the QRS complex was slight. 2. The T waves in all 9 experiments (Lead II and Lead I) became inverted rather than higher (fig. 2). On the other hand, in two dogs out of the three, the Q-T interval was prolonged as in the other experi-

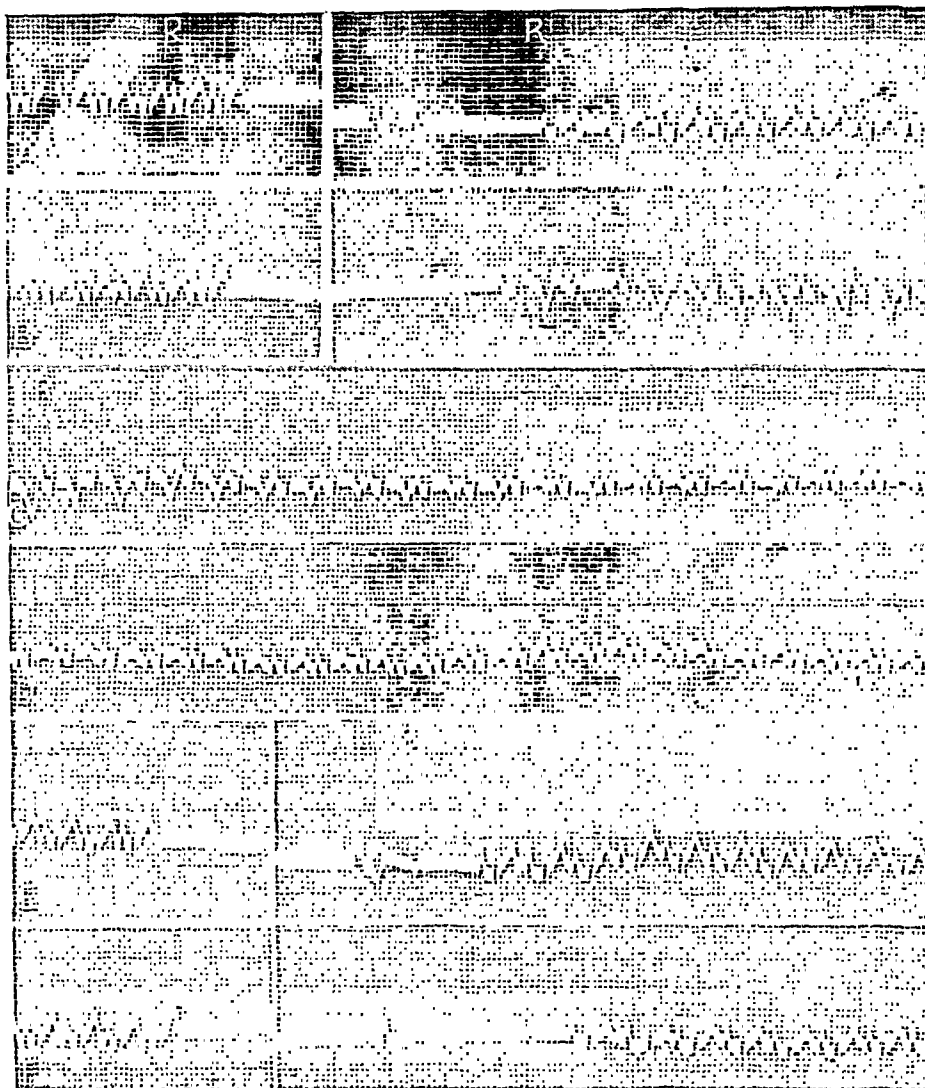


Fig. 2. Catheter openings at base of left ventricle. All Lead II.

Line A: Vagal arrest alone. Between the first and second parts of the record, 4.8 sec. are omitted. During this period no beats occurred. Shortly before the end of vagus inhibition, a single ventricular escape is seen; this T wave is inverted because the area of R is large.

Line B: Vagal arrest; 5 sec. omitted, with no escapes; 50 cc. of Ringer's solution at 1°C. injected during arrest, leading to slight widening of QRS and inversion of T waves.

Lines C and D, continuous with B.

Line E: Vagal arrest; 5 sec. omitted, with one escape; 50 cc. of Ringer's solution at 67°C. injected.

Line F: Control, as in line A; 5 sec. omitted, with one escape. Note that the warm solution prevented most of the T change due to slowing as in the controls.

ments. In the one dog, however, the Q-T prolongation was slight; and in this same dog injection of Ringer's solution at body temperature also caused inversion of T. For this result we have no explanation.

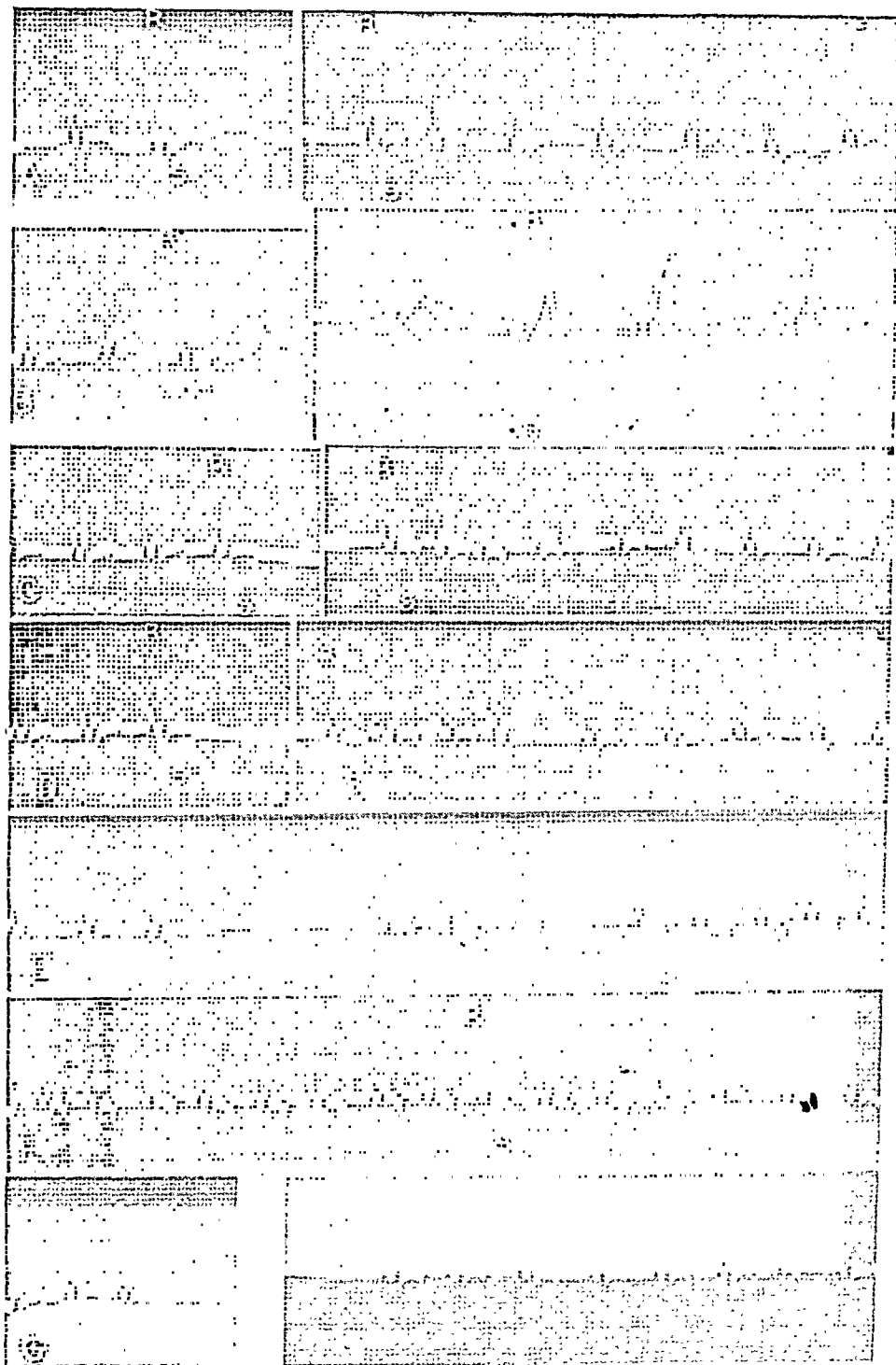


Fig. 3. Right ventricle. Firm rubber catheter between tricuspid leaflets, with opening pointed toward apex.

Line A: Lead II. Vagal arrest, with no injection; 3.2 sec. omitted, with no escaped beats.

*Warming the Inner Surface of the Left Ventricular Apex.* *T-wave changes* (fig. 1). In 7 experiments on 3 dogs (5 precordial and 2 in Lead II) an upright T wave became distinctly lower or slightly inverted with the injection of solutions which entered the syringe at from 65° to 71°C. and in one experiment, 80°C. In the one experiment with Ringer's at 47°C. a clearly visible change of the same sort was produced. The changes produced by warming were much less conspicuous than those due to cooling. Since this fact will not be discussed later, it may here be noted that two things help to account for it. 1. The warm solution was usually 25° to 30° above body temperature; the cold solution was 37 to 40° below. 2. For a 10° temperature change, the effects in living tissues are quantitatively greater in the low, than in the high, temperature range.

*Q-T interval changes.* The Q-T interval was not changed by warming. This is explained in the discussion.

*The QRS complex.* In Lead II, the R wave tended to become lower, but the changes here, as well as in the precordial lead, were slight. In the precordial lead, the R tended to become higher. The S waves were little if at all affected.

*Persistence of the effects.* The changes did not last so long as those due to cooling.

*Cooling the Inner Surface of the Right Ventricle.* In one dog, the opening of the catheter was actually in the right auricle; in the other dog, its open tip was in the ventricle between the A-V valve cusps, and the jet of solution was probably directed toward the apex.

*T-wave changes.* In the 14 experiments, two dogs were used. In Leads II and III and in the precordial lead from near the apex of the right ventricle, there was an increase in T-wave amplitude, and this was just as striking as the change resulting from cooling within the left apex (fig. 3). The effects persisted, with gradual diminution, for over 25 sec. Ringer's solution at body temperature (5 expts.) produced no changes.

Comparison of  $T_2$  and  $T_3$  demonstrates that  $T_1$  would also have been moderately elevated, if it had been recorded.

*Q-T interval changes.* In one dog the increase in the Q-T interval was less striking than in the left ventricular experiments; but it was equally great in the other.

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Line B: Lead II. Taken a few minutes after A; 2.6 sec. omitted, with no escapes; 50 cc. of Ringer's solution at 1°C. injected during arrest. The T gradually decreased to the pre-injection level during at least 25 sec. after the end of this strip. A second vagal control was like A.

Line C: Lead III. Vagal arrest. No injection; 3.2 sec. omitted; no escapes.

Line D: Lead III, a few minutes after C. Vagal arrest; 50 cc. of Ringer's solution injected at 40°C.; 2.0 sec. omitted; no escapes.

Line E: Lead III. Vagal slowing; 50 cc. of Ringer's solution injected at 69°C. No part omitted.

Line F: Continuous with E.

Line G: Lead III. Vagal arrest, a few minutes after F. No injection.

*QRS-complex changes.* In general, the QRS-complex changes were the reverse of those due to left ventricular cooling. In Leads II and III the R wave was much decreased in amplitude, whereas the S wave became much deeper (fig. 3).

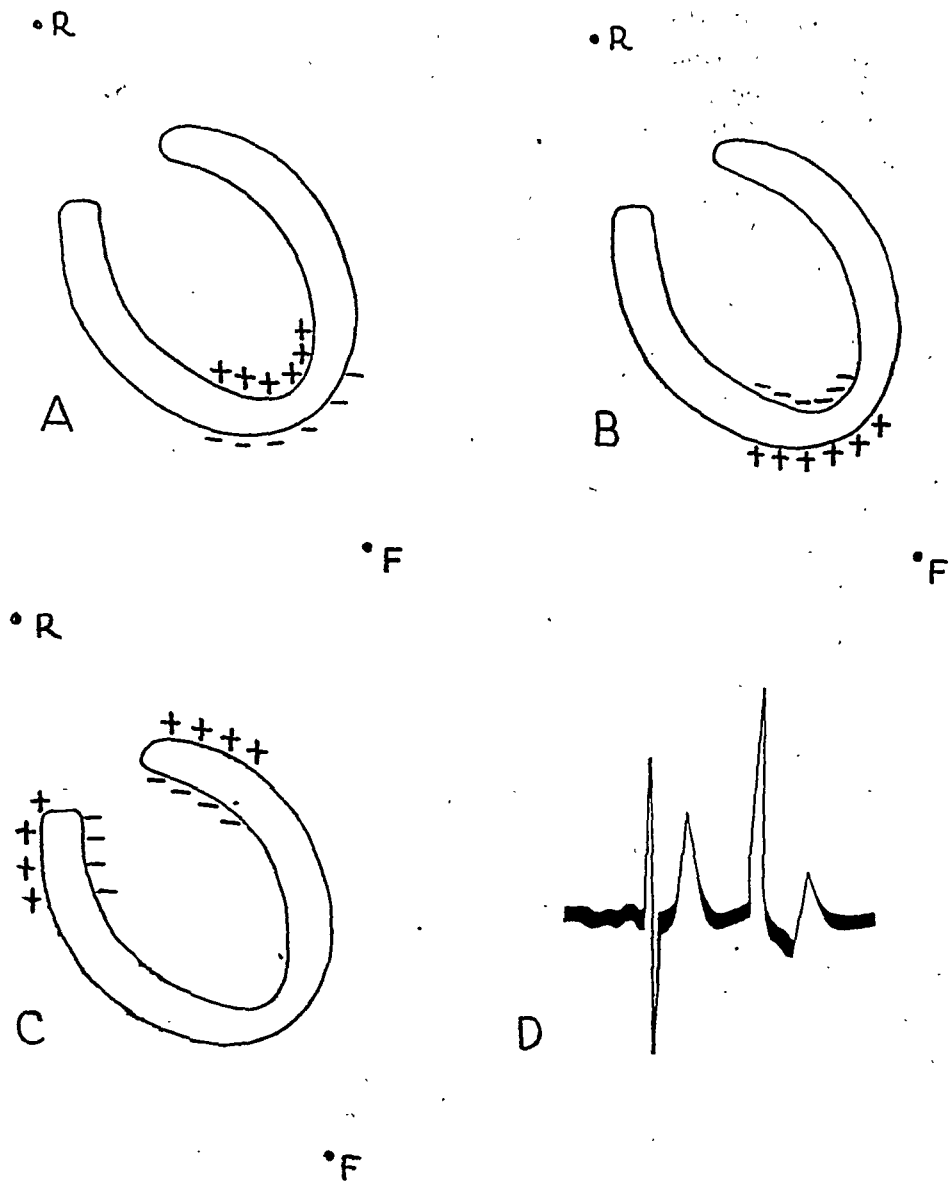


Fig. 4

The widening of the QRS complex was much less conspicuous than in left cooling. In the precordial lead, R was slightly increased and S slightly reduced in amplitude in the one dog so tested.

*Warming the Inner Surface of the Right Ventricle.* This was done in only one dog (fig. 3). The T waves in the 3 experiments were changed from low notched T waves, to diphasic T waves, the initial, downward phase being deeper than the final, upward phase. These effects apparently persisted for over 15 seconds.

The R wave became slightly higher; the S wave less deep.

No changes, except the usual effects of slowing, occurred in control experiments, with or without injection of Ringer's solution at 40°C.

DISCUSSION. In order to make clear the argument involved in the discussion, it is necessary briefly to review certain points of the theory involved in electrocardiographic interpretation. To simplify the discussion, we shall diagrammatically represent only the left ventricle and consider only the T wave. In figure 4 A, the ventricle is shown between R and F. The galvanometer is connected to R (right arm) and F (left leg) in such a manner that relative negativity at R produces an upward deflection in the electrocardiogram. It is well established that a cooled surface, during repolarization when T is inscribed, is negative relative to an uncooled surface. This was recently shown by Ashman, Ferguson, Gremillion and Byer (7), but, of course, it has long been known. Returning to figure 4 A, the epicardial surface at the apex is cooled. The outer surface is negative relative to the inner surface during inscription of the T wave. The T wave, therefore, is inverted, since R is positive and F is negative (11).

In figure 4 B, the endocardial surface at the apex is cooled. The orientation of charges is reversed, and, as in the experiments herein reported, the T wave is upright.

In C of the figure, the inner surface at the base is cooled. During T-wave inscription, R is positive, and F is negative. Hence, the T wave is inverted. This latter interpretation is analogous to the explanation of the S wave, as advanced by Bayley (8).

Diagram B will probably also apply to cooling the inner surface of the right ventricle.

The foregoing simple analysis is adequate to account for the changes in the T waves which are observed when no change appears in the QRS complex. Wilson et al. long ago pointed out that, *ceteris paribus*, any change in the net area of the QRS complex is associated with an equal but opposite change in the T wave (9, 10). The RS-T segment is included as a part of the T wave. In our experiments, the periods of cardiac arrest were often terminated by ectopic ventricular beats, and also, the temperature changes, especially the cooling, produced large changes in the net QRS area. Under such circumstances the T deflection may be a thoroughly unreliable index of the magnitude of the net electrical effect, as projected onto the line of the lead which is used. It is necessary to compare net QRS-T-complex areas. This is well illustrated by figure 4 D, which is a careful copy of two successive complexes obtained during cooling of the right ventricle, and also by figure 2 A and E. The first QRS-T group shows a large, wide, upright T wave. The net QRS-T area was, roughly, 106 microvolt seconds. The second QRS-T group shows the very wide, high, R wave of a ventricular premature beat. The T wave is diphasic, and much reduced in amplitude. Yet the net area of QRS-T is at least 100 microvolt-secs. It is this area we must measure, and not merely the T deflection, since, as Wilson, et al. have demonstrated, this area measures the net electrical effect of differences in time-course of repolarization of different portions of the heart muscle. In this heart, the net QRS-T area before cooling was about 18 microvolt-secs. The difference between this area

and the area found after cooling, i.e., over 100 microvolt-sec., is the magnitude of the change produced by cooling.

In those experiments in which the tip of the catheter was within the ventricular cavity near the apex, the injected Ringer's solution must mix with the blood within the chamber, and, in view of the volume introduced, some blood and solution must be forced out into the aorta, where the pressure must have fallen to a very low level during the six or eight seconds of cardiac standstill. Since the pressure in the aorta is low, it is unlikely that much of the fluid forced out of the ventricle will pass into the coronary circulation during cardiac quiescence. Therefore, it is safe to assume that the temperature of the endocardial ventricular surface will change far more than the temperature of other portions of the muscle. No doubt some temperature change will occur in all layers of the wall which are brought into contact with the injected solution. Possibly, since the apex of the ventricle is thinner-walled than the base, an appreciable change in the temperature of the epicardial surface may have occurred. If so, the change must have been far less than that of the endocardial surface. The experiments of F. M. Smith have demonstrated that cooling the epicardial surface at the apex of the dog's heart brings about inversion of the T waves in Lead II (11), and Hoff and Nahum found in the dog that cooling the outer surface of the left ventricle causes the T waves to become inverted in all limb leads (12). The increase in the height of the T waves which we have found with preponderant cooling of the endocardial surface cannot, therefore, be ascribed to conduction of heat from the epicardial surface, so that it becomes cooler than it is under physiological conditions. The changes in the T waves must, therefore, be due to preponderant cooling of the endocardial surface, especially at the apex. The effects are those which, in theory, should result from endocardial temperature change, and they demonstrate that changes in the electrical state of the endocardial surface of the ventricles may modify the form of the electrocardiogram, and that this modification may be very conspicuous when the endocardial change is great.

The prolongation of the Q-T interval brought about by cooling gives some information concerning the absolute change in the temperature of the endocardial surface. The Q-T is approximately doubled in slowly beating hearts when the temperature is reduced by 10°C. The observed increase in the Q-T ranged from 60 to 90 per cent or more. This suggests a temperature fall of at least 6° to 9°C. It must be remembered that the injected cold solution was mixed with an unknown volume of warm blood. Warming the endocardial surface, on the other hand, had no appreciable effect upon the Q-T interval. This is to be expected, since not all the heart was warmed and those parts of the ventricles which were unchanged in temperature will determine the timing of the end of the T wave.

The changes in the QRS complex were hardly less striking than the changes in the T wave. In lead II, cooling the inner wall of the left ventricle increased the height and width of the R wave. There is good reason to believe that this is due to the decrease in velocity of conduction of the excitation wave, both in the Purkinje fibers and in the cooled portion of the ventricular muscle. Nahum and Hoff have demonstrated that essentially the same effect follows cooling the epicar-

dial surface of the left ventricle (13). It can readily be understood in terms of the analysis of the QRS complex given by Gardberg and Ashman, an analysis which assumed that depolarization of the subendocardial muscle laminae produces potential differences detectable in the limb leads (14). Cooling the inside of the right ventricle slightly reduced the height of the R wave in Lead II and more markedly increased the depth of the S wave. The finding confirms our knowledge that when depolarization of the right ventricle is delayed, the electrical axes which persist after depolarization of the left ventricle have a general upward direction in the vertical heart, such as the dog's (15).

No claim is made that the results of these experiments throw any *direct* light upon the question of the genesis of the usual upright T wave of the human electrocardiogram. They are, however, consistent with the view that the upright T waves may be produced mainly by an endocardial-epicardial difference in rate of repolarization.

#### SUMMARY AND CONCLUSION

The temperature of the endocardial surface of the dog's ventricle was changed by the introduction into the ventricle, by arterial catheterization, of Ringer's solution at various temperatures. Cooling the inside of the left apex changed the form of, and widened, the QRS complex and also caused conspicuous increase in the height and width of the T waves. Warming had opposite, but less striking, effects on the T wave, and changed the form of the QRS complex. Ringer's solution at body temperature caused no changes. In other experiments, cooling the inner surface of the right ventricles caused similar T wave changes, but reverse effects on the form of the QRS complex. Cooling the basal left endocardial surface caused inversion and usually widening of the T waves.

These experiments have demonstrated that procedures, the preponderant effect of which should be to change the temperature of the endocardial ventricular surface, produce T-wave changes of the kind which theoretical considerations have led us to expect. It is reasonable to suppose, therefore, that the electrical effects were, in fact, brought about by a change in rate of repolarization of the endocardial surface. If this conclusion be justified, it follows that changes in the electrical state of the subendocardial muscle surface play a part in the genesis of the electrocardiogram.

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## THE EFFECT OF THE INHALATION OF HIGH AND LOW OXYGEN CONCENTRATIONS ON RESPIRATION, PULSE RATE, BALLISTOCARDIOGRAM AND ARTERIAL OXYGEN SATURATION (OXIMETER) OF NORMAL INDIVIDUALS<sup>1</sup>

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The experiments to be described here were designed primarily to determine the threshold of the normal human respiratory and circulatory systems to anoxemia. In addition, data were also obtained which related to other basic problems in respiration and circulation: the magnitude and variability of response of large numbers of normal men to inhalation of low oxygen mixtures, the existence of tonic activity of the chemoreceptors of the carotid and aortic bodies in man, the effect upon respiration and circulation of brief exposures to 100 per cent oxygen and the correlation between decreases in arterial oxygen saturations and changes in respiratory minute volume, pulse rate and cardiac output.

Attempts have been made previously to measure the slightest decrease in inspired oxygen tension which will produce measurable physiological changes in man. Lutz and Schneider (1) and Ellis (2) stated that inhalation of 18 per cent oxygen is stimulant to human respiration while Boothby's data (3) suggest that the threshold is not reached until 16 per cent oxygen is breathed. Each of these studies yielded valuable information with respect to conditions that might obtain in airplanes but none supplied accurate data regarding thresholds of basal subjects breathing constant low oxygen concentrations. Most of their experiments were conducted with non-basal subjects sitting in a low pressure chamber. Some were rebreathing experiments in which constant concentrations were not maintained and respiration was measured in 5 minute units. In others, respiratory minute volume was not measured but was inferred from alveolar CO<sub>2</sub> measurements.

Since the desired data could be secured only by great attention to detail, our method is presented fully.

<sup>1</sup> This work was performed under a contract approved by the Committee on Medical Research between the Office of Scientific Research and Development and the University of Pennsylvania.

**METHOD.** Reliable respiratory data are difficult to obtain in normal man because breathing can be regulated voluntarily and is often disturbed by many factors in the environment. We avoided the use of the low pressure chamber as a method for producing anoxia because of the attendant noise, excitement and apprehension, and because of actual discomfort that may arise from the ears or gastrointestinal tract at reduced ambient pressures. Lutz and Schneider had previously noted (1): "Some men dreaded going into the low pressure chamber; others disliked the mouthpiece and nose clip of the rebreather . . . The subjects showed some degree of anxiety or excitement. In all experiments in which an attempt was made to detect the earliest effects of low oxygen, the psychic factor had to be considered."

Our experiments were planned to minimize this important and often neglected subjective factor. Subjects were chosen who were familiar with laboratory surroundings and with the experimenters. Each rested comfortably on a bed or ballistocardiograph 45 to 60 minutes before control observations were made. It has been demonstrated by Soley and Shock (4) that minute volume of respiration becomes steady in 20 minutes, but in our experience stabilization of pulse rate requires a longer period. The room in which measurements were made was quiet and an effort was made to afford a reassuring atmosphere by permitting ordinary conversation by the laboratory personnel. Pulse rates were counted at the radial artery for 30 seconds each minute. Blood pressure determinations were abandoned when it became apparent that the subjects' respiratory tracings were disturbed by periodic inflation of the cuff. A mouthpiece and nose clip were not used because, though these provide for minimal dead space, they become uncomfortable after a short time. Instead a very small rubber mask, covering just the nose and mouth, was fixed to the face with rubber cement. In this way, annoying head straps and undesirable dead space were avoided. The mask was comfortable throughout the experimental period and no accumulation of CO<sub>2</sub> was noted. Expired air was conducted through an "11.2 rubber valve" and measured in a balanced compensating spirometer. Inspired gases were breathed through a special demand valve designed to operate with minimal inspiratory resistance. The demand valve was connected to a manifold to which were attached 6,000 liter high pressure tanks of 100 per cent oxygen and 20.9, 18, 16, 14.5, 12, 10 and 8 per cent oxygen in nitrogen. A shift to any desired oxygen concentration could be made quickly and noiselessly without the subject's knowledge. Arterial oxygen saturation was measured by an automatically compensating oximeter (5) placed on the subject's ear. Stroke volume and cardiac output per minute were calculated from ballistocardiographic tracings by the area method (6).

Thirty-three subjects breathed gas mixtures in the following order: room air (8 min. ), 100 per cent O<sub>2</sub> (8 min.), room air (8 min.), 18, 16 or 14.5 per cent O<sub>2</sub> (8 min.), 100 per cent O<sub>2</sub> (6 min.), room air (8 min.), another of the 18, 16 or 14.5 per cent O<sub>2</sub> mixtures (8 min.) and 100 per cent O<sub>2</sub> (8 min.). Oximeter readings always reached a plateau within 4 minutes after shift to these low oxygen mixtures. The data obtained during each experimental period were checked by the following procedure. The *increase* in respiration and circulation that

occurred when the subject breathed a low oxygen mixture following room air was compared with the rapid *decrease* when the anoxemia was terminated abruptly by high oxygen inhalation. Thus we were able to detect "drifts" in circulation or respiration that might have occurred in the 8 minute period of anoxia such as increases in these functions due to discomfort or slight decreases due to further attainment of the basal state. Unless otherwise stated the data obtained from the switchback to 100 per cent oxygen was quantitatively similar to that obtained during the shift from room air to the low oxygen mixtures.

Forty-eight other subjects were studied by less accurate methods. Some of these subjects were not completely basal though they had been resting at least 15 minutes prior to control readings. Seventeen subjects breathed room air, 18, 16, 14.5, 12 and 10 per cent oxygen in succession, each for 8 minutes. Sixteen subjects breathed room air, 16, 14.5, 12 and 10 per cent oxygen successively, each for 8 minutes. Two subjects breathed room air and then 10 per cent oxygen. Two others breathed room air and then 16 and 10 per cent oxygen. Five breathed room air, then 16 and 8 per cent oxygen and six breathed room air and 8 per cent oxygen only. The 8 per cent oxygen was breathed for only 4 to 8 minute periods. Twenty-one of these individuals wore a standard full face army gas mask and 27 wore an aviation type half mask. The gas mask had a dead space of 400-500 cc. and the half mask a dead space of approximately 200 cc. Analyses of the atmosphere within the gas mask showed a CO<sub>2</sub> percent ranging from 3.2 to 4.1 at the end of expiration and from 0.5 to 2.0 per cent at the end of inspiration. We have not used any of the data obtained upon these subjects in our determination of threshold data. However, we felt justified in using the data obtained during inhalation of 12, 10 and 8 per cent oxygen to define the magnitude and variability of response of normal man to anoxemia.

**RESULTS.** 1. *Immediate effect of breathing 100 per cent oxygen on respiration and circulation.* It is believed by some that the chemoreceptors of the carotid and aortic bodies are active at the oxygen tension (90-100 mm. Hg) prevailing in the arterial blood when the subject is breathing room air at sea level (7, 8, 9). Others feel that these end organs do not respond until a moderate degree of anoxia is produced (10). Surgical removal of the chemoreceptors has not been performed in man, but one can at least estimate the degree of activity of oxygen sensitive chemoreceptors by a "physiological denervation". The inhalation of 100 per cent oxygen probably accomplishes such a denervation for the following reason: If the carotid and aortic bodies of a normal man breathing room air were activated by the oxygen tension existing in arterial blood, this should be a factor in the maintenance of normal respiration and circulation. Inhalation of 100 per cent oxygen, by raising arterial oxygen tension to 670 mm. Hg should abolish this chemoreceptor activity. Since the major function of the chemoreceptors is the response to lowered oxygen tension, removal of this component of chemoreceptor activity should depress respiration and circulation. Oxygen inhalation, of course, does not remove whatever tonic discharge might result from receptors activated by the normal acidity, carbon dioxide tension, and temperature of arterial blood.

Thirty-three subjects were studied during the change from room air to 100

TABLE 1  
*Immediate response to 100 per cent oxygen (1-2 min.)*

SUBJECT	MINUTE VOLUME (LITERS/MIN)		PULSE RATE (PER MINUTE)	
	R.A.	100%	R.A.	100%
50	6.1	6.6	78	76
51	7.2	7.2	68	64
52	7.3	7.0	68	64
54	6.8	7.0	66	64
55	5.1	4.4	76	76
56	7.0	6.3	84	84
57	8.8	8.1	76	72
58	7.2	8.9	60	60
59	8.9	7.7	72	72
60	9.0	7.2	108	96
61	6.4	6.8	84	88
70	6.4	7.0	76	76
71	6.6	7.0	68	64
73	10.8	10.5	54	50
74	6.4	6.0	72	70
75	4.6	4.4	74	70
76	7.0	7.0	64	60
77	6.5	6.5	64	64
78	9.2	7.2	60	58
79	9.0	8.5	80	72
80	7.6	7.0	62	64
81	7.8	7.5	72	72
82	7.5	7.5	64	60
83	8.4	7.8	84	78
84	6.6	6.8	76	70
85			76	72
86	8.6	7.2	86	84
87	9.6	9.2	60	60
88	11.0	10.6	52	48
89	10.0	10.6	76	76
90	5.8	5.4	78	76
91	7.6	8.5	82	80
92	7.4	7.1	64	56
Aver.....	7.6	7.4	72.2	69.6
Aver. difference R.A. to O <sub>2</sub> .....		-0.23		-2.66
St. dev. (difference).....		0.74		3.10
St. error mean (difference).....		0.13		0.54
P .....		0.066		<0.001

R.A. = Lowest reading of last two minutes during inhalation of room air.

100% = Lowest reading of first two minutes during inhalation of 100% O<sub>2</sub>.

per cent oxygen. The results are indicated in table 1. Average respiratory minute volume was depressed 3.1 per cent and pulse rate 3.7 per cent during the first two minutes of 100 per cent oxygen inhalation. The probabilities of ob-

taining these changes by chance are 0.066 for respiration and less than 0.001 for pulse rate. If the data are examined individually marked variations can be observed from subject to subject. Four subjects showed no change in respiratory minute volume, 9 showed an increase (maximum, 23 per cent in subject 58) and 19 a decrease (maximum, 22 per cent in subject 78);<sup>2</sup> in 6 of the 19, the decrease in minute volume amounted to 10 per cent or more. Two subjects responded with a rise in pulse rate; in 9, the pulse rate was unchanged while in 22, it decreased. The maximal decrease in pulse rate was 12 per minute. Twenty-eight subjects responded with a decrease in either pulse rate or respiratory minute volume, but only 13 had decreases in both functions.

*Comment.* These figures indicate that an increase in oxygen tension from that normally present in arterial blood (90–100 mm. Hg) to approximately 670 mm. Hg produced a small immediate decrease in respiratory minute volume or pulse rate in 28 of 33 subjects. If this change can be attributed wholly to a functional denervation of oxygen sensitive receptors in the carotid and aortic bodies, these data suggest that some chemoreceptors are tonically active at the oxygen tension present in the arterial blood of many normal men breathing room air at sea level. The small degree of this change indicated that they are only minimally active, since an increase in oxygen tension of approximately 570 mm. Hg was followed by only a 3.1 per cent average decrease in respiratory minute volume. It must be remembered that a stimulant effect of oxygen (11) which tends to increase respiration may be acting simultaneously to limit the extent of this immediate depression of minute volume. Since this stimulant action of oxygen does not appear to extend to pulse rate, pulse rate changes probably represent the true extent of tonic activity that the chemoreceptors exert upon the circulation.

It is unlikely that the effect of oxygen inhalation will ever be determined in humans before and after surgical chemoreceptor denervation. Therefore, the evidence can only be presumptive in favor of tonic chemoreceptor activity as far as man is concerned. Watt, Dumke and Comroe (12) noted a depression of respiration immediately following the inhalation of oxygen by unanesthetized, trained dogs; this effect was no longer present when the carotid and aortic bodies were denervated. Similar effects might reasonably be expected to occur in man. If removal of all activity of the carotid and aortic bodies (i.e., responses to changes in  $p\text{CO}_2$ , pH and temperature as well as to  $p\text{O}_2$ ) could be accomplished in man, a greater degree of tonic activity might be demonstrable.

2. *Threshold of the respiratory and circulatory systems to low oxygen.* Fourteen subjects were exposed to 18 per cent oxygen, 20 to 16 per cent oxygen (table 2), and 11 to 14.5 per cent oxygen under the conditions outlined under Methods.

<sup>2</sup> Watt, Dumke and Comroe (12) performed similar studies upon 11 adults and reported that the usual immediate (1–2 min.) effect of breathing 100 per cent oxygen was an increase in respiratory minute volume. Their subjects were not in a basal state, were breathing through a mouthpiece (with nose clipped) from Douglas bags and into a wet test gas meter. The present experiments were designed to eliminate the undesirable features of their technique; we feel that the present method is far more accurate in detecting small changes in respiration.

TABLE 2

*Threshold of respiratory and circulatory systems to reduction in oxygen concentration of inspired air*

A—18% O<sub>2</sub>

SUBJECT	MINUTE VOLUME EXPIRED AIR (LITERS/MIN.)				PULSE RATE PER MIN.			
	R.A.*	18%*	18%†	100%†	R.A.*	18%*	18%†	100%†
59	8.3	7.6	7.2	7.3	70	70	68	72
60	7.7	7.8	7.6	7.5	96	96	92	92
61	6.0	7.2	6.8	6.5	88	88	84	72
70	8.0	7.4	6.9	6.6	80	88	88	84
71	6.5	5.7	5.8	5.6	66	72	72	60
73	11.2	10.7	10.6	10.4	52	56	54	50
74	7.4	6.7	6.5	6.2	73	75	73	68
75	4.9	5.3	4.8	4.5	70	72	70	66
76	7.3	7.0	7.0	7.0	62	68	64	62
77	9.2	9.5	8.8	9.2	67	73	70	64
78	8.0	8.5	8.0	6.8	59	64	62	56
79	8.3	7.9	7.7	8.0	80	82	81	70
80	7.9	7.6	7.5	6.2	59	61	60	56
81	7.8	8.0	8.0	7.6	68	70	70	68
Aver.....	7.7	7.6	7.4	7.1	71	74	72	67

B—16% O<sub>2</sub>

	R.A.	16%	16%	100%	R.A.	16%	16%	100%
50	8.1	8.5	8.4	7.8	72	84	84	76
51	7.5	7.9	7.7	7.7	64	66	64	60
52	7.3	7.0	7.2	7.7	64	64	64	64
54	7.3	7.6	7.6	6.2	68	72	72	60
55	5.6	5.6	5.2	5.0	76	82	80	72
56	6.2	6.4	6.2	4.6	84	94	92	80
57	9.0	9.2	8.6	7.0	76	84	84	68
58	8.6	9.7	9.7	7.5	56	68	68	56
59	8.2	8.6	8.0	7.7	72	82	80	68
60	7.8	8.1	8.3	7.0	88	96	96	80
61	6.5	7.3	6.8	6.7	84	90	88	72
70	7.2	7.5	7.4	5.0	81	84	86	78
71	5.3	7.4	7.0	5.0	66	76	76	62
73	11.0	10.8	10.8	10.8	53	56	56	50
74	7.3	7.6	6.0	6.5	76	76	74	68
75	4.9	6.1	5.9	4.7	70	75	74	64
76	7.8	8.1	8.1	8.0	64	73	70	64
77	8.8	9.0	8.2	9.0	73	77	75	64
78	8.0	8.8	8.2	7.5	60	65	64	60
79	8.0	8.5	8.2	7.5	80	82	82	68
Aver.....	7.5	8.0	7.7	7.0	71	77	76	67

\* Average of last three minutes of inhalation.

† Lowest figure of last two minutes of low oxygen and first two minutes of 100% oxygen.

TABLE 3

*Change in respiration and circulation produced by breathing O<sub>2</sub> or O<sub>2</sub>-N<sub>2</sub> mixtures at 6 to 8 minutes*

ROOM AIR TO	RESPIRATION (LITERS/MIN.)	RESPIRATIONS PER MINUTE	PULSE PER MINUTE	% O <sub>2</sub> SATURATION (OXIMETER)
100% O <sub>2</sub> (33 sub- jects)				
Aver.....	+0.6	+0.2	-4.5	+4.0
Range.....	-2.3 to +3.1	-3.3 to +3.3	-12.0 to +4.0	+1.0 to +7.0
$\sigma$ diff.....	1.0	1.4	3.6	1.5
P.....	<0.01	0.8	<0.001	<0.001
18% O <sub>2</sub> (14 subjects)				
Aver.....	-0.1	0.0	+3.2	-2.5
Range.....	-0.7 to +1.2	-1.0 to +1.0	0.0 to +8.0	-1.0 to -4.5
$\sigma$ diff.....	0.6	0.6	2.6	0.9
P.....	0.5	1.0	<0.001	<0.001
16% O <sub>2</sub> (20 subjects)				
Aver.....	+0.5	+0.4	+6.0	-5.0
Range.....	-0.3 to +2.1	-2.0 to +2.0	0.0 to +12.0	-2.0 to -7.0
$\sigma$ diff.....	0.5	1.0	3.8	1.6
P.....	<0.001	0.1	<0.001	<0.001
14.5% O <sub>2</sub> (11 sub- jects)				
Aver.....	+0.5	-1.1	+4.0	-8.8
Range.....	-0.9 to +1.1	-9.0 to +3.0	0.0 to +11.0	-3.0 to -11.5
$\sigma$ diff.....	0.6	3.5	3.2	4.1
P.....	0.05	0.4	<0.001	<0.001
12% O <sub>2</sub> (32 subjects)				
Aver.....	+0.5	+0.3	+11.7	-17.0
Range.....	-1.7 to +2.2	-6.0 to +10.0	0.0 to +22.0	-10.0 to -28.0
$\sigma$ diff.....	1.0	0.5	6.9	4.8
P.....	<0.01	0.6	<0.001	<0.001
10% O <sub>2</sub> (36 subjects)				
Aver.....	+1.3	0.0	+21.0	-24.5
Range.....	-0.6 to +6.3	-4.0 to +8.0	0.0 to +46.0	-13.0 to -44.0
$\sigma$ diff.....	1.4	2.5	10.0	6.6
P.....	<0.001	0.9	<0.001	<0.001
8% O <sub>2</sub> (11 subjects)				
Aver.....	+6.0	+2.0	+19.0	-25.3
Range.....	+1.7 to +12.9	-2.0 to +9.0	+4.0 to +32.0	-19.0 to -34.0
$\sigma$ diff.....	3.2	3.5	10.9	5.7
P.....	<0.001	0.1	<0.001	<0.001

Eleven of 14 subjects (79 per cent) showed increases in pulse rate ranging from 2 to 8 beats per minute while they were breathing 18 per cent oxygen. At 16 per cent oxygen, 18 of 20 subjects (90 per cent) showed a pulse increase and at

14.5 per cent, 10 of 11 (91 per cent) had pulse acceleration. All of these changes are highly significant statistically (table 3). None of the 33 subjects had a decrease in pulse rate while breathing 18, 16 or 14.5 per cent oxygen. These figures were checked by the switchback to 100 per cent oxygen which was followed by a decrease in pulse rate in 42 of the 45 cases (93 per cent). The average decrease upon breathing 100 per cent oxygen in each case was approximately equal to the increase in rate while breathing the low oxygen mixtures (after an appropriate correction had been made for the decrease in pulse rate which normally occurs when 100 per cent oxygen follows inhalation of room air).

The respiratory responses to 18 per cent oxygen were less consistent than were the changes in pulse rate. The respiratory minute volume of 8 subjects decreased and that of 6 subjects increased when 18 per cent oxygen was breathed following inhalation of air; the average change in minute volume of respiration was a decrease of 0.1 liter per minute. The switchback to 100 per cent oxygen indicated that the respiration of two other subjects had probably increased when 18 per cent oxygen was inhaled. At the 16 per cent level, the minute volume of 17 of 20 subjects (85 per cent) increased, while that of 2 decreased and one was unchanged; the average change was an increase of 0.5 liter per minute. At 14.5 per cent oxygen, minute volume increased in 8 of 10 subjects, the average increase being 0.5 liter per minute. The respiratory changes at 16 per cent and 14.5 per cent oxygen are statistically significant.

There were few marked changes in cardiac output (ballistocardiograph) at 18, 16 or 14.5 per cent oxygen. Three subjects breathing 18 per cent oxygen, one breathing 16 per cent oxygen, and three breathing 14.5 per cent oxygen showed increases in cardiac output exceeding 10 per cent; only two of these increases exceeded 15 per cent, these being increases of 24 and 30 per cent in two subjects breathing 14.5 per cent oxygen.

*Comment.* Since it appears that the chemoreceptors usually are slightly active at the oxygen tension in arterial blood of normal men breathing room air at sea level, it is incorrect to refer to a "threshold" of oxygen tension below normal at which the chemoreceptors first respond. We can discuss, however, the change in oxygen concentration of inspired air below 20.93 per cent that is required to produce any measurable increase in respiration and circulation.

Increase in pulse rate occurred in 79 per cent of the subjects breathing 18 per cent oxygen. Since 19 per cent and 20 per cent oxygen in nitrogen were not employed in these experiments, we are unable to state whether circulatory stimulation would have occurred at these higher concentrations. It is evident that an increase in pulse rate is a more sensitive index of anoxemia than an increase in respiratory minute volume. Of 14 subjects breathing 18 per cent oxygen, only 6 showed an increase in minute volume of respiration, the percentage increase being 1.3, 2.6, 3.3, 6.3, 8.2 and 20 per cent. Two additional subjects showed decreases of 15 and 17.3 per cent when they were shifted from 18 per cent to 100 per cent oxygen, and these may be considered properly to have shown respiratory stimulation while breathing 18 per cent oxygen (which could have been masked by extraneous factors). Therefore 3 of the 14 subjects showed increases

in minute volume exceeding 10 per cent of control values and 5 showed increases in excess of 5 per cent. It must be concluded that the respiratory system is not stimulated significantly in most normal men by a change from 20.93 to 18 per cent oxygen, though the circulatory system is. It is unlikely that the respiratory response to anoxia was limited by a decline in alveolar and arterial  $p\text{CO}_2$  (8); in order to reduce alveolar or arterial  $p\text{CO}_2$  in these subjects it would be necessary that breathing rate or depth be increased and this was not the case in most subjects. Furthermore in studies performed by Shock and Soley (14) the respiratory response that could be attributed to anoxia was not increased by addition of  $\text{CO}_2$  to the low oxygen mixtures. The response of the respiratory center to anoxemia therefore does not appear to be reduced by decreased arterial  $p\text{CO}_2$  incident to hyperventilation; such experimental data is at variance with the theoretical observation of Gray (15). At 16 per cent oxygen, respiratory stimulation was more apparent. Seventeen of 20 subjects exposed to that concentration responded with some increase in minute volume and in 5 of these the increase was 10 per cent or greater. Sixteen per cent oxygen appears to be a concentration which just produces respiratory stimulation in the majority of normal men. It should be pointed out that the so-called "threshold" level is an average figure; some individuals show no measurable increase in respiration even when breathing 10 per cent oxygen.

Ellis (2) in 1919, using a rebreathing technique with absorption of expired  $\text{CO}_2$ , stated that respiratory minute volume increased in 22 of 29 subjects when the oxygen concentration in the rebreather tank was 18.1 per cent. This figure of 18.1 per cent was not actually determined but was obtained by measuring the oxygen concentration in the rebreather at the end of the experiment and plotting the oxygen reduction backwards as a straight line, a practice in common use at that time in the Air Service Medical Research Laboratory. His subjects were not basal, were never in a constant state of anoxia and respiration was measured only as 5 minute averages. Lutz and Schneider (1) performed a series of experiments both in the low pressure chamber and with the Dreyer nitrogen apparatus and concluded that increased breathing first occurred at about 656 mm. Hg atmospheric pressure, which is equivalent to breathing 18 per cent oxygen at sea level. Close inspection of the only experiments in which they actually measured respiratory minute volume shows no definite increases until the barometric pressure was reduced to 560 mm. or the equivalent of breathing 15.2 per cent oxygen at sea level (their table 5) or until altitudes of 3,000 to 18,000 feet had been reached, the equivalent of 18.6 per cent to 10 per cent oxygen breathed at sea level (their table 6). Soley and Shock (14) measured respiratory minute volumes in 18 normal men each of whom breathed 17 per cent oxygen on two occasions. No information is given regarding the number of minutes of each anoxic period or of the oxygen saturations reached. Seven of the 18 had some increase in respiration on both trials but only 3 of the 36 trials resulted in increases of more than 10 per cent. The average increase in the 36 trials was 0.1 per cent. Their data suggest that respiration was stimulated consistently in only one of the 18 subjects breathing 17 per cent oxygen.

Boothby (3) has presented data on the composition of alveolar air of large numbers of subjects during short exposures to decreased barometric pressure. At 1,000 feet altitude, the control alveolar  $p\text{CO}_2$  of 186 subjects was 36.7 mm. Hg (average). At a simulated altitude of 6,000 feet the mean alveolar  $p\text{CO}_2$  had fallen to 35.4 mm. Hg in 50 individuals. It would appear that respiratory stimulation began above 6,000 feet altitude which corresponds approximately to inhalation of 16.6 per cent oxygen at sea level. It should be emphasized that wide individual variation occurred in this series.

On the basis of our data and of the only other data obtained upon basal individuals breathing constant low oxygen concentrations (14), it can be concluded that circulation is usually stimulated by reducing the inspired oxygen percentage from 20.9 to 18 but that respiration is stimulated in only a few individuals breathing 18 or 17 per cent oxygen, and is not increased in the majority until 16 per cent oxygen is inhaled. These findings in man are consistent with the estimate of chemoreceptor activity in cats given by Von Euler et al. (16), employing carotid body action potentials as the indicator of response to anoxia.

3. *Response of normal men to 12, 10 and 8 per cent oxygen.* Pulse rate increased in 96 per cent, 97 per cent and 100 per cent of subjects at the 12, 10 and 8 per cent oxygen concentrations respectively while respiratory minute volume increased in 59, 81 and 100 per cent of subjects at these same levels. The pulse rate increased not only more consistently but also to a greater extent at the 12 and 10 per cent levels than did respiration (table 3). Ballistocardiographic studies were not made at these levels. At the 8 per cent oxygen level, respiratory minute volume increased markedly for the first time (average 63 per cent). The average respiratory rate did not change until 8 per cent oxygen was breathed; the increase in respiration at all oxygen concentrations was achieved largely by an increase in depth of breathing.

Individual variation in respiratory response was marked. Seven of the 36 subjects showed no respiratory stimulation even at 10 per cent oxygen, although 4 of these responded to the switchback to 100 per cent oxygen with a decrease of more than 10 per cent. In those individuals whose respiration increased in response to inhalation of 10 per cent oxygen, the percentage increase varied from 2 to 66 per cent. Although all subjects responded to 8 per cent oxygen with an increase in respiratory minute volume, the increases ranged from 15 to 150 per cent.<sup>3</sup>

*Comment.* The two most characteristic features of the respiratory response to anoxia (the slight changes until 10 per cent or 8 per cent oxygen are inhaled and the wide individual variations) are shown in figure 1, which is a composite

<sup>3</sup> In three subjects, deliberately excluded from this study, an unusual respiratory response was noted during and immediately after the inhalation of 10 or 8 per cent oxygen. This response was characterized by a marked increase in respiratory rate and depth which progressed to alarming proportions. A sudden shift to 100 per cent oxygen did nothing to alter this reaction, the hyperpnea continuing for a few minutes with marked fixity of posture on the part of the semi-conscious subject. The mechanism of this response, which is obviously different from the ordinary respiratory reaction to anoxia, is uncertain.

picture of our data upon respiratory minute volume at 100, 20.9, 18, 16, 14.5, 12, 10 and 8 per cent oxygen, Soley and Shock's at 17 per cent oxygen and 12 per cent oxygen (14) and those of Horvath et al. (17) at 6.0, 5.2 and 4.2 per cent oxygen.

It appears that the reactivity of the human respiratory system to oxygen lack depends primarily upon the absolute level of oxygen tension rather than upon any definite number of millimeters change in tension. For example, a change of approximately 570 mm. Hg. oxygen tension in inspired air (from 100 per cent oxygen to room air) may change respiratory minute volume only about 3 per cent. An additional decrease of 22 mm. Hg (20.9 to 18 per cent oxygen) may produce no change and another of 15.2 mm. Hg (18 to 16 per cent oxygen) an increase of only 7 per cent in respiratory minute volume. On the other hand a similar change of 15.2 mm. Hg (from a level of 10 per cent to 8 per cent oxygen) produced an increase of 46 per cent in respiratory minute volume. The similarity of this activity curve (see fig. 1) to the oxygen dissociation curve has been commented upon by Irving (18).

The response of the circulatory system to anoxia differs markedly from the respiratory reaction. As shown in figure 2, pulse rate increases with the smallest decrement in oxygen percentage of inspired air and the pulse increases progressively as the oxygen concentration is further reduced. This circulatory response may be due to direct stimulation of the vasomotor center in addition to activation of the chemoreceptors by anoxia. It is likely that the vasomotor center is stimulated more and/or depressed less by anoxia than is the respiratory center. The relative importance of the central and reflex components in the circulatory response to anoxia has never been demonstrated clearly but the relatively greater resistance of the vasomotor center as compared to the respiratory center is known. For example, the local ischemia of increased intracranial pressure will stimulate the central vasomotor mechanism at a time when the respiratory center is no longer able to function. In addition the circulation is often relatively spared by narcotics which markedly depress respiration. That anoxia may act in similar fashion can be inferred.

Our experiments indicate that oxygen sensitive chemoreceptors in many men are tonically active, as stated by Bernthal (8) and by Heymans (9). However so far as respiration is concerned, they have only minimal activity under normal conditions at sea level and their activity increases very little until dangerously low oxygen concentrations are breathed, thus partly supporting the opinion of Schmidt and Comroe (10) that they are primarily emergency mechanisms brought actively into play only during times of stress.

4. *Delayed effect of inhalation of 100 per cent oxygen.* In addition to measuring the immediate effect of an increased arterial oxygen tension, we also recorded the alterations in respiration and circulation produced at the end of 6 to 8 minutes of high oxygen in the same 33 subjects (table 3).

Respiratory minute volume increased (average 7.6 per cent), while pulse rate and cardiac output per minute decreased by 5.5 and 8 per cent respectively. All of these changes are highly significant statistically. The reduction in cardiac

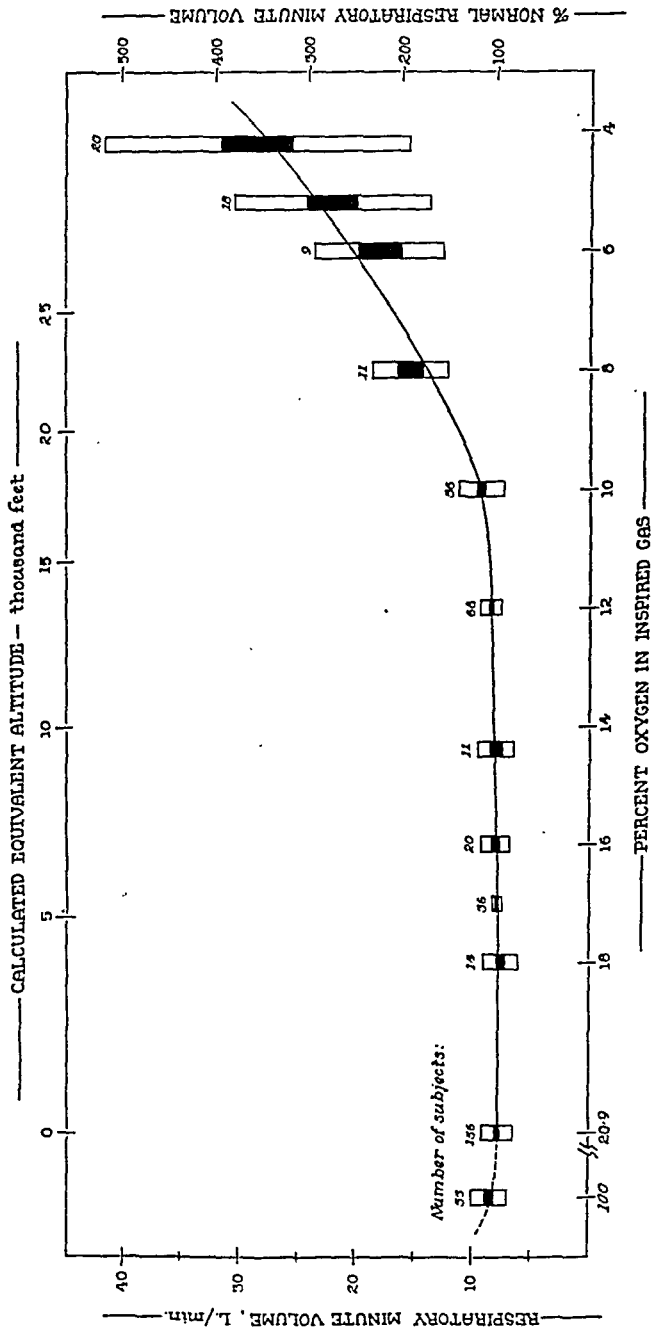


Fig. 1. The effect of inhalation of various low oxygen mixtures upon respiratory minute volume. The data of Shock and Soley for 17 and 12 per cent  $O_2$  (14) and of Horvath et al. for 6, 5.2 and 4.2 per cent  $O_2$  (17) are included. The solid bars represent one standard error on each side of the mean. The open bars represent one standard deviation on each side of the mean. The smoothest possible curve is drawn through the solid bars.

output was caused largely by a reduction in pulse rate rather than in stroke volume.

*Comment.* The stimulant effect of inhalation of 100 per cent oxygen upon respiratory minute volume has been described previously (11) (12) (29). It has been attributed (20), *a*, to increase in tissue  $p\text{CO}_2$  caused by the decreased availability of reduced hemoglobin for  $\text{CO}_2$  transport; *b*, to increase in medullary  $p\text{CO}_2$  brought about by cerebral vasoconstriction; *c*, to a lower respiratory tract reflex initiated by the irritant effect of oxygen, and *d*, to improvement in arterial oxygen tension at the medullary center. It is now known that inhalation of 100 per cent oxygen decreases cerebral blood flow in man about 10 per cent; however, this vasoconstriction is not associated with a significant increase in the  $p\text{CO}_2$  of internal jugular blood (21). Of the four possibilities listed above, the third has more factual supporting evidence, for it is known that inhalation of 100 per cent oxygen for 4 to 20 hours leads to clinical signs of pulmonary irritation and it is

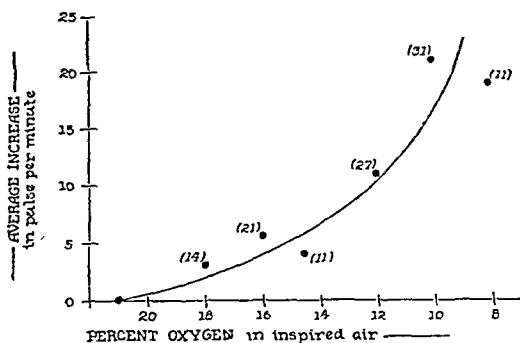


Fig. 2. Effect of inhalation of various low oxygen mixtures on pulse rate. The numbers in parentheses refer to the number of subjects.

likely that sensitive receptors in the lower respiratory tract could respond at once to such an irritant.

The increased respiration, bradycardia and decreased cardiac output noted in this study correspond with those of Otis et al. (29) rather than with the larger changes noted by Whitehorn et al. (19).

5. *Correlation of oximeter readings with respiratory minute volume and pulse rate during inhalation of various low oxygen mixtures.* Until 1945 it had been customary to regard 95 per cent as the degree of saturation of hemoglobin in the arterial blood of normal man breathing room air. Recent data indicate that this figure may be too low at least in some individuals (22, 23, 24). Our "room air setting" was therefore arbitrary; in early experiments it was 96 per cent and in later experiments it was 98 per cent. As decreasing concentrations of oxygen were breathed oximeter readings decreased. The average readings noted when the subjects had reached a relatively steady state during exposure to each of the low oxygen mixtures used were as follows: 18 per cent oxygen: 94, 16 per cent oxygen: 91, 14.5 per cent oxygen: 89, 12 per cent oxygen: 81, 10 per cent oxygen: 73, 8 per cent oxygen: 72 (see fig. 3). These average figures agree within 2 per cent saturation with those obtained by Millikan in a large series which included direct determinations of oxygen saturations on arterial blood (25).

These are only average figures, however, and it should be noted that there was a wide range of readings at each level. The extent of this spread is illustrated in figure 3, where it can be seen for example, that at 10 per cent oxygen, one individual's oximeter had decreased by 13 per cent saturation (saturation of 85 per cent) from the room air setting while another's had fallen 44 points to reach a saturation of 50 per cent.<sup>4</sup>

*Comment.* This wide range in arterial oxygen saturations occurring in individuals breathing the same gas might be explained on the following basis: Those with a high oximeter reading may have had the most marked increases in respiratory minute volume and those with low oximeter readings may have had minimal respiratory responses to low oxygen. Under anoxic conditions hyperventilation is known to increase oxygen saturation (26). Examination of our

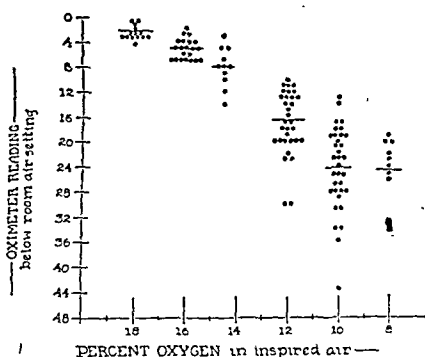


Fig. 3. Effect of inhalation of various low oxygen mixtures on arterial oxygen saturation (oximeter). The numbers in parentheses refer to the number of subjects.

data, however, affords little correlation between the degree of respiratory response to anoxia and the level of oximeter readings. However, the respiratory responses in our subjects were rarely of great magnitude. There was much better correlation between oximeter readings and pulse rates than between oximeter readings and respiratory minute volume or depth of breathing (see figs. 1, 2, 3).

Barach (27), Pruitt (28) and Houston (26) have shown that arterial saturation cannot be predicted from knowledge of the inspired oxygen concentration. Our data confirm these findings. Clinical applications of anoxia (such as the anoxemia test for coronary insufficiency) should be based therefore on arterial oxygen saturation rather than on a inhalation of fixed percentage of inspired oxygen.

#### CONCLUSIONS

The respiratory and circulatory responses of 81 normal individuals to the inhalation of 100 per cent  $O_2$  and 18, 16, 14.5, 12, 10 or 8 per cent  $O_2$  in nitrogen have been studied.

1. An immediate decrease in pulse rate or respiratory minute volume occurred in 28 of 33 subjects when inhalation of 100 per cent oxygen followed room air.

<sup>4</sup> We did not make repeated observations of ear thickness and are therefore unable to state whether ear thickness values changed after the original period of vasodilatation due to heat. The possibility of a decrease in ear thickness as the result of vasoconstriction secondary to anoxia might be considered, but Millikan (5) considers such a sequence of events unlikely.

This suggests that some chemoreceptors of the carotid and aortic bodies are tonically active at the oxygen tension present in the arterial blood of many normal men breathing room air at sea level.

2. An increased minute volume of respiration was noted in only a few individuals breathing 18 per cent oxygen, the majority showing no significant change until 16 per cent oxygen was inhaled. The respiratory response to anoxia was characterized by extreme individual variability and by the fact that only slight stimulation was noted until 10 or 8 per cent oxygen was inhaled.

3. The response of the circulatory system to anoxia differed markedly from the respiratory response. Significant increase in pulse rate occurred when the concentration of oxygen in the inspired air was reduced from 20.9 to 18 per cent. Progressive increases in pulse rate accompanied further reduction in inspired oxygen concentration.

4. Additional evidence of the respiratory stimulant action of 100 per cent oxygen was obtained. Bradycardia and a reduction of cardiac output per minute were noted simultaneously under these conditions.

5. The wide range in arterial oxygen saturation described by others for individuals breathing the same low oxygen mixture was confirmed.

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## FURTHER STUDIES ON THE ACUTE EFFECTS OF INTRA-ABDOMINAL PRESSURE<sup>1</sup>

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The preliminary observations on abdominal distention reported by Booker and Johnson in 1944 (1) pointed out that death could be caused in animals by maintaining a constant distention of the abdomen either by inflating a balloon which had been surgically inserted into the abdomen or by injecting air into the abdomen (pneumoperitoneum). It was emphasized in that communication that the circulatory failure produced resulted from a venous stasis with consequent loss of plasma into the abdomen and the usual events to follow. It was recognized early in this work that it mattered not whether air was introduced into the stomach, intestine or peritoneal cavity. In all instances, death was produced, sooner or later, by maintaining an increased intra-abdominal pressure.

It occurred to us that perhaps abdominal size might play a part in the immediate and late blood pressure response observed upon inflation of the abdomen and that abdominal size might influence the time that animals could withstand given pressures before passing into circulatory failure and death. The work was, therefore, extended to determine the relationship between abdominal size and the ability to withstand a given intra-abdominal pressure; the amount of intra-abdominal pressure necessary to cause changes in blood pressure and respiration and the effect of increasing the intra-abdominal pressure on the heart (ECG).

**PROCEDURE.** Eleven dogs were used in these acute experiments. The dogs varied as to overall weight and builds as well as to sex and age. All of the animals were anesthetized with Tuinal<sup>2</sup> used intravenously (approximately 40 mgm/kgm). After anesthesia the abdomen of the animals was measured carefully in centimeters by means of a calibrated tape or flexible rule, as shown in figure 2. The carotids, vagi, and trachea were isolated and a tracheotomy performed followed by introduction of a cannula connected through an ether bottle top with a tambour for recording respiration. One carotid (usually the left) was cannulated and connected to a mercury manometer for constant blood pressure recording. Usually the femoral artery and vein on the right side were isolated just below Poupart's ligament. If further anesthesia was necessary during the experiment, the anesthetic was introduced via the femoral vein. In some cases the blood pressure was also taken via the femoral artery by the

<sup>1</sup> Supported by a grant from the Rockefeller Foundation to Howard University School of Medicine.

<sup>2</sup> Furnished through the kindness of the Ely Lilly Company, to whom we express thanks and appreciation.

use of a mercury monometer. The femoral vein was also used to measure venous pressure at intervals by the introduction of an 18-gauge needle connected to a manometer in which chloroform or oil was used. A mercury manometer connected to an 18-gauge needle inserted into the peritoneal cavity was used for continuous measurement of the intra-abdominal pressure.

In several of these experiments the intra-thoracic pressure was measured by inserting a glass cannula into the fifth intercostal space and after suturing tightly, connecting it by rubber tubing to a mercury manometer. In addition to the intra-tracheal measurement of respiratory excursion, there was also measurement of thoracic respiratory movements by means of a pneumograph tied in some instances around the upper thoracic and in other instances around the lower thoracic wall. The intra-abdominal pressure was increased by means of a

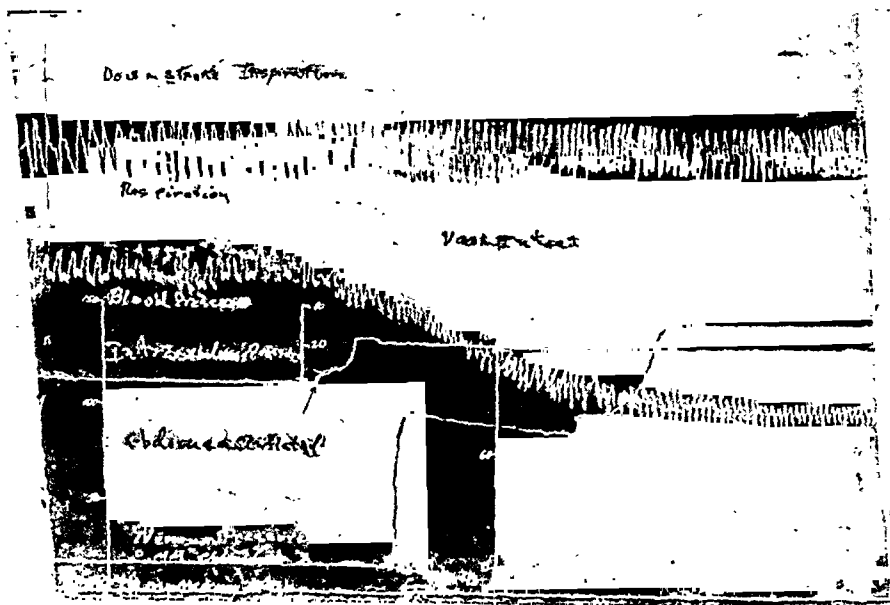


Fig. 1. Showing increase in venous pressure and drop in arterial pressure following the increase in intra-arterial pressure. Intra-abdominal pressure was raised 20 mm. mercury.

tire pump connected through pressure tubing to an 18-gauge needle which was inserted into the peritoneal cavity. The pressure within the abdomen was easily maintained or increased at will by this means. Prior to and after anesthesia hematocrits were done in some cases by use of Van Allen tubes and centrifuging. Also blood samples were taken on occasions from arteries (femoral or carotid) and veins (femoral or jugular) to measure oxygen as well as carbon dioxide content by means of the Van Slyke manometric method. Several of the dogs' circulation times were measured by means of injection of sodium cyanide into the femoral vein and accurately timing the period between injection and appearance of respiratory rate increase. A series of ECG's was done at regular intervals, using three leads and the chest leads.

**OBSERVATIONS AND DISCUSSION.** Figure 1 bears out the point mentioned in the preliminary report that increasing the intra-abdominal pressure causes an

increase in the venous pressure. The arterial pressure, however, may either fall immediately or rise to fall after several minutes. We have observed a tendency of the arterial pressure in dogs of small or medium sized abdominal areas (fig. 2) to fall immediately upon inflation, while the arterial pressure of dogs having larger abdominal areas seems first to rise, then fall (fig. 3). In one or two instances the arterial pressure remained increased above the normal

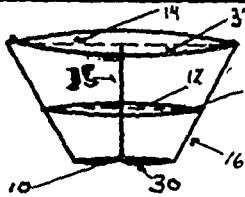
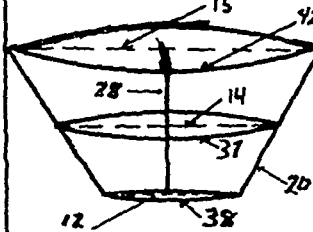
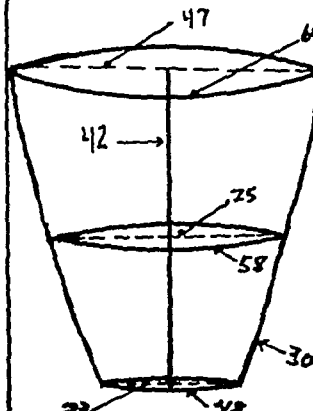
SIZE	DIAGRAM	Appx. Abdom. Press.	B. P. RESPONSE	FAILURE AND DEATH
Small		10-30 mm. Hg.	Definite decrease after early attempt to recover downward trend occurs	45 Min. to 1½ hours
Medium		10-30 mm. Hg.	Same as above	1 hour to 1:45 min.
Large		40-80 mm. Hg.	Usually increase at first inflation. May remain increased for sev. min. then fall or may remain increased for hrs. (2-3) and collapse suddenly.	2 - 3 hours

Fig. 2. Diagram of typical abdominal sizes worked with, response to increased intra-abdominal pressure, and length of time elapsed before circulatory failure and death. Measurements are reported in centimeters.

following tremendous increases—beyond 50-80 mm. of mercury. We are at a loss to explain why abdominal size seems to influence this differential arterial pressure response while venous pressure rises in all cases studied. We recognize that we have not excluded experimentally the possibility that difference of abdominal tone in different planes of anesthesia could be a factor here. We made the attempt, however, to put all animals in plane 2 of surgical anesthesia and maintain them at that plane throughout the experiment. It is of interest to note that only small amounts of intra-abdominal pressure (5-10 mm. mercury)

are necessary to cause a response (either decrease or increase) in the arterial pressure or the venous pressure. We have observed also that increasing the intra-abdominal pressure causes the same drop in the arterial pressure taken at the carotid and at the femoral simultaneously. We can believe, therefore, that at least the drop in arterial pressure, following inflation of the abdomen, is of common cause—a decrease in the output of the heart, since the pressure falls, both below the point of inflation and above it.

Raising the intra-abdominal pressure creates a strain on the processes of respiration. First of all, the abdominal muscles, prominently used in the dog at inspiration, become less and less functional as the pressure is increased. In the second place, the diaphragm becomes more and more displaced toward the

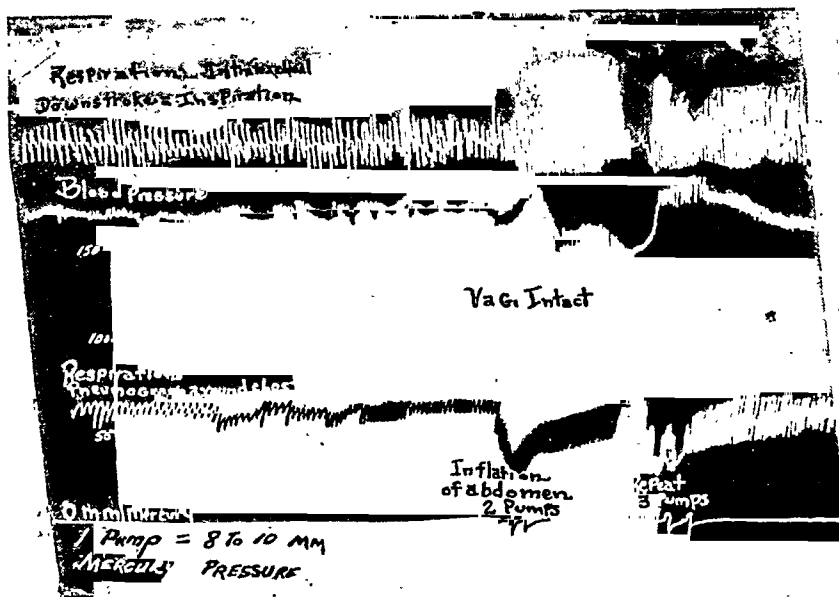


Fig. 3. Showing the response of a dog with large abdominal area to increases of intra-abdominal pressure (see fig. 2), and the increase in thoracic activity following increases in intra-abdominal pressure. Note: Five pumps approximate 50 mm. mercury.

thorax as pressure is increased, tending to decrease the intra-thoracic space (raising the pressure). So that now the thoracic musculature tends to work harder (fig. 4) for three reasons: 1. Abdominal muscles, which are active at inspiration in raising the intra-abdominal pressure in order to force blood to the heart, are no longer active since they can not overcome the tremendous positive pressure in the abdomen. 2. As a result of (1) the thorax, which ordinarily increases in negative pressure at inspiration, now no longer has the aid of the abdominal muscles in facilitating the sucking effect on the venous blood at inspiration, and there is, therefore, a tendency of the thorax to compensate for the absence of its abdominal collaborator. 3. The displaced diaphragm tends to reduce the thoracic expansion at the phrenic margin and slightly above so that the middle and upper thorax becomes hyperactive. These factors all sum to contribute to the venous stasis in the abdomen, created first by the marked

maintained positive-pressure. That the available oxygen to the tissues is markedly reduced is evident by an average drop of from 16.5 volumes per cent to 2 volumes per cent in arterial blood by the end of the first hour of inflation.

Our attention was called to the work of Duomarco, Rimini and Recarte (2), who have correlated increased abdominal pressure with pulmonary emphysema and congestive heart failure in patients. They found that in both pathological conditions the intra-abdominal pressure was increased; that emphysematous patients and chronic asthmatic patients show a marked hypertrophy of abdominal musculature; that chronic cardiaes (especially those greatly emphysematous) develop large abdominal hernias. They believe, following experimental work on dogs, that the increase in intra-abdominal pressure seen in patients mentioned above is due to increase in intra-thoracic pressure which invariably causes a thoracic venous hypertension, displacing the diaphragm toward the

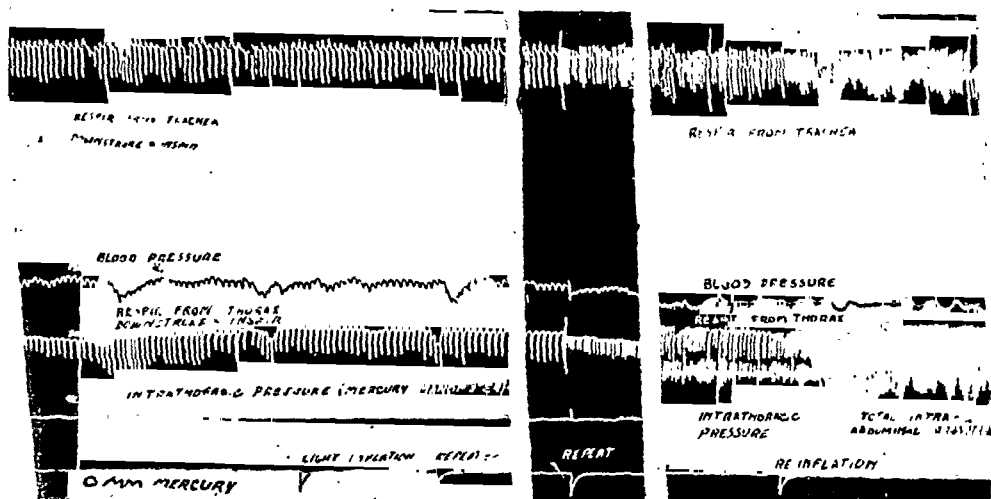


Fig. 4. Showing changes in intra-thoracic pressure and in intra-thoracic expansion as intra-abdominal pressure is increased. Pressure (intra-abdominal) was increased 6 mm. at each inflation. Note base line changes which caused intra-thoracic breathing and intra-thoracic pressure excursions to become superimposed upon each other.

abdomen and raising the intra-abdominal pressure. Harris and Chillingworth (3) have also shown that increasing the intra-thoracic pressure creates an emphysema in dogs. Luckhardt (4), Holt (5), Young (6) and others have independently shown that raising the intra-thoracic pressure increases the venous pressure. Our work, however, is just the reverse to that of Duomarco, Rimini and Recarte and others mentioned above since the abdominal pressure is raised and the effect is seen on the thorax. These pieces of work although attacked from opposite directions show further the close relation of the abdominal and thoracic pressures related to respiration and circulation and how sensitive the processes of respiration and circulation are to changes in pressure within the abdominal and thoracic cavities.

How much the heart is affected by increased intra-abdominal pressure can not be assayed. Certainly an extensive shift in the diaphragm causes a shift in the mediastinum, which may cause axis deviation. We were interested,

however, in determining whether or not a coronary and subsequent ventricular conductive involvement could be seen via the vagal pathway, described by Booker (7) in liver traction experiments. It is significant in the electro-cardiograms (fig. 5) that in leads two and three the QRS complex is reduced by half (and in some instances only 2 mm. high) in 15, 30 and 45 minute periods following inflation. After section of the vagi the QRS complex tended to recover in lead 2, but not in lead 3. It appears that not only left axis deviation occurs, but the

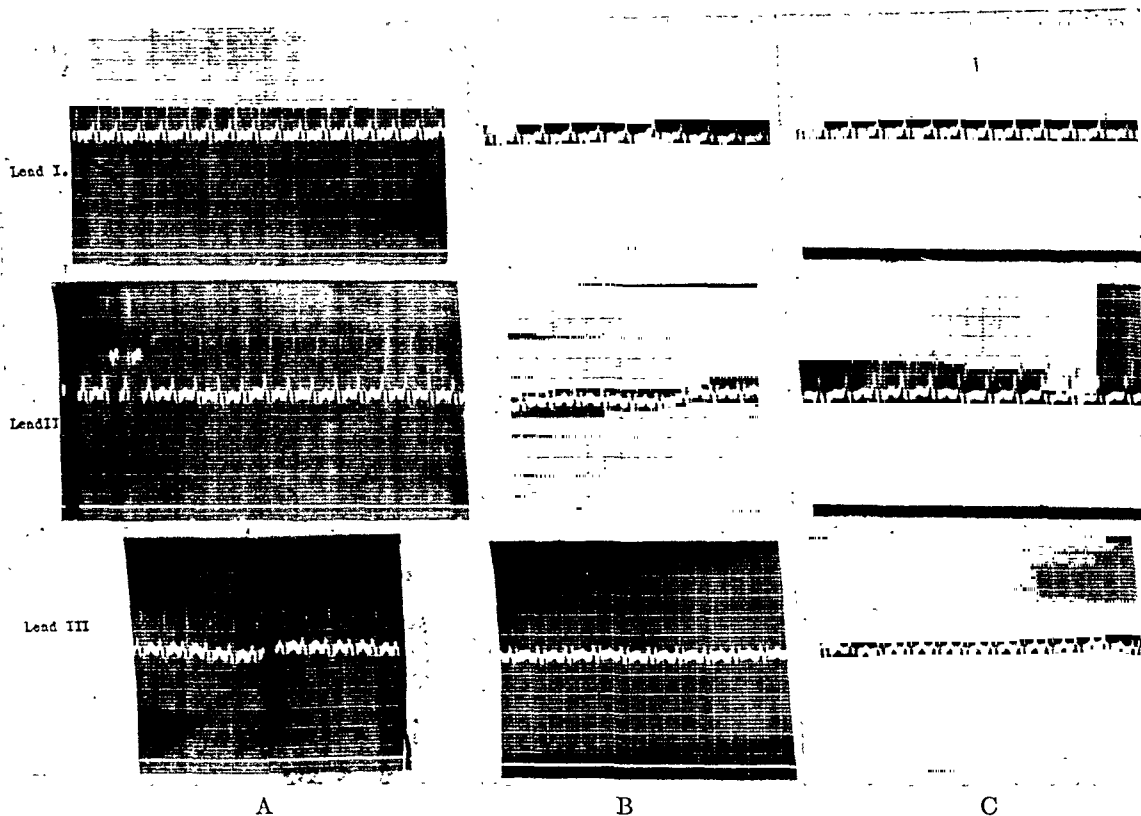


Fig. 5(a). Preinflation (anesthesia).

Fig. 5(b). After 45 minutes of inflation.

Fig. 5(c). After sectioning vagi. Animal had been inflated 55 minutes.

low voltage and notching of the QRS complex in lead 2 (45 min. after distention) seem to indicate that a disturbance in conduction over the ventricular muscle exists.

Our impression is that we are dealing with the effect of distention on the coronary circulation referred to by Fenn, LeRoy and Gilbert (8), who found that not only distending the stomach and gall bladder decreased coronary blood flow, but air injected into the peritoneal cavity did likewise. Atropine or vagotomy abolished this reflex.

#### SUMMARY AND CONCLUSIONS

1. Experiments have been described attempting to correlate abdominal size with the ability of animals to withstand given intra-abdominal pressures;

showing the effects of intra-abdominal pressure on thoracic pressure and on thoracic breathing, and showing the effects of increased intra-abdominal pressure on the heart.

2. The evidence seems to show that animals having small abdominal areas are more sensitive to small increases in intra-abdominal pressure (5–10 mm. Hg), usually showing a definite preliminary drop and subsequent down hill course in the arterial pressure when the intra-abdominal pressure is increased; while animals having larger abdominal areas require a higher intra-abdominal pressure to cause a change in arterial blood pressure, which may first rise and remain increased for several minutes (or longer), in some cases remaining increased for one to two hours, only to collapse suddenly.

3. A given pressure seems to cause an earlier circulatory failure and death in animals with small abdominal areas than the same pressure in animals of larger abdominal areas.

4. Without exception increasing the intra-abdominal pressure causes an increase in thoracic respiratory activity, most likely due to the inactivity of the abdominal muscles. The upper thorax is more active than the lower thorax, which we attribute to pushing of the diaphragm into the thorax as a result of the increased intra-abdominal pressure, making more difficult the contraction of intercostal muscles in the lower thorax.

5. We have suggestive evidence that increased intra-abdominal pressure causes a damage to the myocardium, which we ascribe to narrowing of the coronaries via the vagal pathway. The tendency of the QRS complex to return toward normal following sectioning of the vagi lends weight to our postulation.

6. We reiterate the point suggested in the preliminary report, that the distended stomach or intestine increases the total intra-abdominal pressure the same as free air in the peritoneal cavity.

The authors are desirous of expressing their sincere thanks to Dr. J. B. Johnson, Head of the Department of Medicine, for the use of his electrocardiograph and Mrs. Jewel Mazique for her technical assistance in its operation. We wish also to express our thanks to Dr. Beverly Graves, of this Department, for his helpfulness and suggestions during the last of these experiments and to Dr. Riley Thomas, Department of Medicine, for his aid in interpretation of the electrocardiograph.

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# THE RESPONSE OF SMOOTH MUSCLE TO STRETCH

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The high sensitivity of visceral smooth muscles to stretch is well known and probably plays a rôle in the regulation of the normal activity of this type of muscle. In the present paper, the manner in which stretching can elicit responses will be studied by recording electric potential changes.

**METHOD.** Action potentials were recorded by a d.c. amplifier and a G. E. oscillograph. The ureter of the dog was used. Because of the possibility of movement artifacts, longitudinal stretching is impractical. Instead, the muscle was stretched by raising the pressure inside, distending chiefly the circular fibers. For this purpose the pelvic end of the isolated ureter was attached to a glass cannula which was connected to a pressure bottle and a water manometer. Pressure could be applied suddenly by turning a stopcock. Provided that the mouth of the cannula was wide enough and not filled with fluid, the organ was inflated by air practically instantly. At the low pressures generally used, only the region nearest the cannula was distended. One lead was attached to this region, the other about a centimeter away. The preparation was placed inside a moist chamber which was immersed in a constant temperature bath. A temperature of 32° to 35° was usually chosen because under these conditions no spontaneous contractions occurred and because the slow potentials could be distinguished more readily from the conducted responses. The inflation with air caused only a slight and brief movement artifact or none at all.

**RESULTS.** Below a certain pressure, which varies under different conditions, distention produces no electric change. Above threshold, distention elicits a slow transient potential change, the distended region becoming more negative. The magnitude of this response increases with the pressure applied. That it is a local response is shown by the fact that it is monophasic and that it can be graded, whereas the conducted responses strictly follow the all-or-none relation regardless of whether they are elicited by stretch or electric stimulation (2). At a still higher pressure a conducted response arises from the local response. The local potential change is the steeper, and the delay in the onset of the conducted response the briefer, the higher the pressure used for stretching the muscle (fig. 1).

In skeletal muscle, stretching produces potential changes purely physically (7). No evidence for such an effect could be found in the experiments described here.

In some muscles, distention induced oscillatory local potentials followed by a burst of conducted responses. These potentials are exactly like those which may precede spontaneous discharges (3) and probably only occur in slightly damaged tissue (fig. 2).

If one end of the ureter is distended continuously by moderate pressure, a long series of conducted responses is discharged at rather regular intervals. At constant pressure, the frequency gradually declines within the first minute but becomes fairly constant later on.

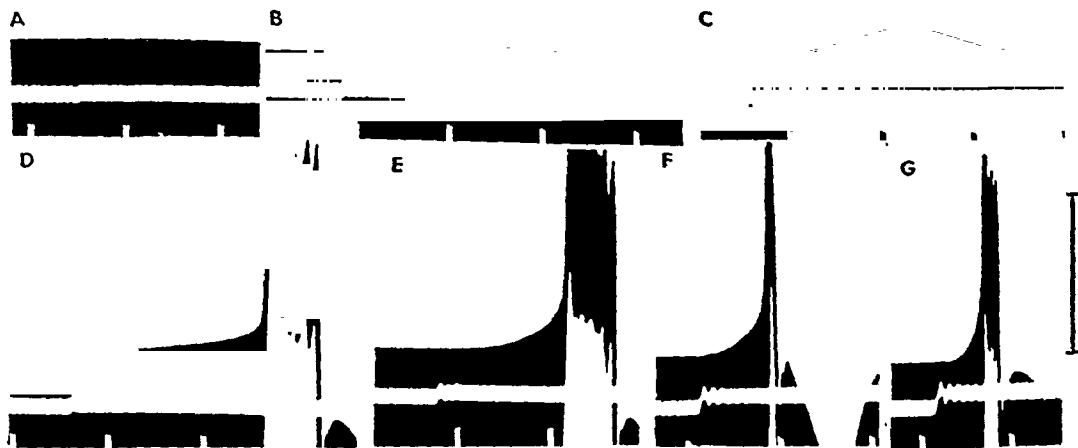


Fig. 1. Potentials produced by distending the ureter with air. The signal shows the moment of application of pressure. The pressures were: in A, 3.5; in B, 4.2; in C, 5.8; in D, 4.7; in E, 11; and in G, 13 cm. water. In A the pressure was below threshold; in B and C, local responses, the distended regions becoming more negative; in D, E, F, and G, the local responses were followed by conducted responses. Their onset was the faster, the greater the pressure. The threshold for conducted responses is rather variable, as seen by comparing C and D, and is higher for about 30 seconds after a previous response. Temp. 34°. Time, 1 second. Calibration, 1 mV.

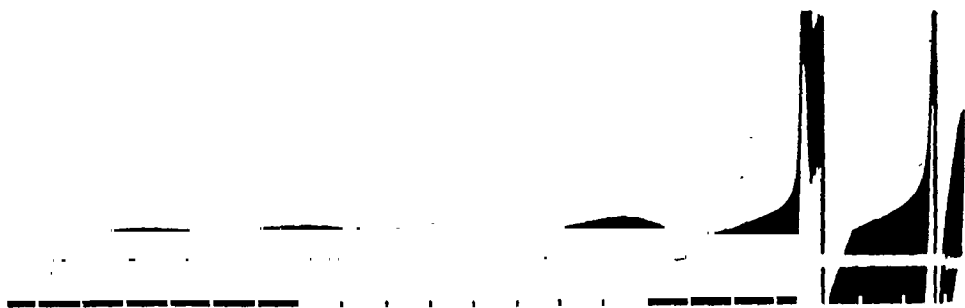


Fig. 2. Oscillatory potential of the ureter produced by distention. A burst of conducted responses, each arising from the peak of a local potential (only 2 are shown) is induced. Temp. 33°. Time, 1 second.

These experiments simulate rather closely the function of the ureter under normal conditions. The flow of urine probably controls the frequency of peristaltic movements by distending the pelvic end of the organ. Even at the low temperatures chosen in the experiments described here, distention by a pressure of less than 5 cm. water often induced responses. It is probable that the sensitivity is appreciably higher under normal conditions.

The observation that a local response is the first effect of stretching gives

additional support for the purely muscular origin of the peristaltic contractions. The magnitude of the local potentials (0.2 mVolt), their slow onset and monophasic character leave no doubt that they are a muscular response and not due to a discharge of nervous elements. It should be noted also that other experiments have shown that the local potentials are accompanied by a weak contraction (3).

The local potentials which are induced by stretching are exactly like those which underlie the automaticity of the muscle and which have likewise been shown to precede spontaneous discharges of cardiac muscle (4) and of nerve fibers (1).

#### SUMMARY

Sudden distention of a region of the ureter with pressure above threshold produces a slow local response like that preceding spontaneous contractions. This response varies with the force applied and, if sufficiently strong, induces a conducted all-or-none response. The greater the pressure applied, the steeper is the local potential change and, therefore, the shorter the delay in the onset of the conducted response. The observation that the latter is always preceded by a local muscular response is further evidence for the muscular origin of the activity of visceral smooth muscle.

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# EFFECT OF LOCAL COOLING ON FLUID MOVEMENT, EFFECTIVE OSMOTIC PRESSURE AND CAPILLARY PERMEABILITY IN THE FROG'S MESENTERY.

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The swelling which occurs when the human hand (1) or the rabbit's foot (2) is exposed to low temperatures without freezing is indirect evidence that capillary permeability is increased by cold, at least in mammals. The increase in permeability to fluid and protein described for the human hand at temperatures between 10° and 3°C. has been attributed to cellular injury (1, 3, 4). It is known, however, that poikilothermic animals (5) and even tissue cultures from man and other mammals (6) can survive exposure to temperatures near the freezing point without suffering apparent harm. It seemed desirable therefore to study, by methods more direct than can be used for experiments with human subjects, the effects of severe local cooling on the capillary endothelium of a poikilothermic animal.

Fluid movement and capillary blood pressure were measured in single capillaries of the frog's mesentery by micromanipulative methods (7, 8). It was found that local cooling to temperatures between -2° and +2°C. reduced the "filtration constant" of the capillary wall and increased the effective osmotic pressure of the blood, even though the concentration of plasma proteins remained the same. These findings in the frog are in direct contrast to the injurious effects of cold in mammals and are discussed with reference to *a*, changes in capillary permeability which result from cooling, and *b*, possible thermosmotic effects.

**MATERIAL AND METHODS.** Frogs (*Rana pipiens*)<sup>2</sup> were pithed in the brain only. During dissection, bleeding was kept to a minimum by using electrocautery. The intestine and mesentery were delivered through a generous incision in the abdominal wall so that the mesentery could be spread gently over a transparent stage about 1 cm. in diameter. For the experiments at room temperature, this was a lucite or glass disc, and when the mesentery was to be cooled, the stage was a similarly shaped box made of  $\frac{1}{4}$  mm. celluloid sheeting through which fluid could be pumped at desired rates from a reservoir containing ice and brine. A continuous very slow drip of Ringer's solution at room temperature kept the exposed tissue moist; the frog's skin was covered by cotton moistened with tap water. Preparations made in this way remained normal in

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<sup>2</sup> The microinjection experiments were performed at the Marine Biological Laboratory at Woods Hole between July 10 and August 24, 1945, using frogs recently shipped from Vermont. They were kept in a shaded place where they could moisten themselves under dripping water but were never immersed.

appearance for many hours. The maximum duration of these experiments was 3 hours. For illumination daylight was used after passage through colloidal gold filters to diminish infra-red radiation. Room temperature ranged from  $21\frac{1}{2}^{\circ}$  to  $25\frac{1}{2}^{\circ}\text{C}$ .

A thermocouple was sealed within the celluloid chamber, just beneath the surface supporting the mesentery. In calibration experiments, temperatures recorded by galvanometer from this position were  $3^{\circ}$  to  $7^{\circ}\text{C}$ . lower than those measured by a junction placed immediately beneath the mesentery. It was not convenient to have such a superficial junction in place during micromanipulation. Owing to variations in the thickness of the mesentery, the overlying layer of Ringer's solution, the room temperature and the rate of circulation of brine within the cooling chamber, the gradient between chamber and mesentery was not entirely constant. In general the temperature of the mesentery was about  $5^{\circ} \pm 2^{\circ}\text{C}$ . higher than that of the chamber.

Rates of filtration and absorption of fluid were estimated according to the technique described by Landis (7). One end of a long, unbranched capillary was gently obstructed with a blunt glass rod. By observing the rate at which the trapped erythrocytes moved away from, or toward, the open end of the minute osmometer thus formed, and by measuring the dimensions of the capillary by ocular micrometer, it was possible to calculate the approximate rate at which fluid was filtered or absorbed in terms of cubic micra per square micron of capillary wall per second. Since fluid movement decreased progressively as equilibrium was approached after obstruction, the rate of movement at the instant of obstruction was obtained by plotting a curve showing the rate of corpuscular movement and constructing a tangent to it at zero time. Measurements were confined to vessels between 765 and 1220 micra in length. If blood flow was not resumed immediately after release of obstruction, it was assumed that injury had occurred, and the observation was discarded.

The apparatus and methods used for the direct measurement of blood pressure in single capillaries were also essentially the same as those described by Landis (8). The vessel was blocked by the glass rod before inserting the micropipette. The level of the fluid in the water manometer was raised or lowered until fluid neither entered nor left the pipette, erythrocytes again being used as indicators. Capillary blood pressure in the vessel under observation could be determined easily to within 1 or 2 mm. of water pressure.

Errors may arise from spontaneous changes in capillary pressure occurring between the time at which the rate of fluid movement is estimated and that at which the pipette is inserted to measure capillary blood pressure. For this reason, pressure was always determined as quickly as possible after the first procedure. Rates of blood flow in the same or parallel capillaries were observed before and after each measurement in order to detect large vasomotor changes.

At the conclusion of each experiment, between 1 and 2 cc. of blood were drawn from the aortic bulb into a heparinized syringe. An ultrafiltrate of plasma was prepared by means of a B-D Swinny filter and a double layer of cellophane under a pressure of about 3 atmospheres; the filtrate thus obtained was protein-

free by sulfosalicylic acid test. Specific gravities of plasma and of ultrafiltrate were measured by Linderstrøm-Lang gradient tube (9). Approximate plasma protein percentages were then calculated, substituting the observed specific gravity of the ultrafiltrate for the value 1.0070 in the formula of Moore and Van Slyke (10).

RESULTS. a. *Capillary permeability before and after freezing: qualitative observations.* In preliminary observations, 6 mesenteries were cooled at various rates until frozen. Rates of blood flow and the appearance of the vessels did not change until temperature fell to within 1 or 2 degrees above the freezing point. Blood flow then became sluggish, and erythrocytes tended to stick to the capillary walls. Crystallization appeared suddenly but not until the temperature of the chamber had fallen to between  $-10^{\circ}$  and  $-15^{\circ}\text{C}$ . (mesentery about  $-5^{\circ}$  to  $-10^{\circ}\text{C}$ .), after periods of cooling totalling  $4\frac{1}{2}$  to 58 minutes. The circulation of cold brine through the chamber was stopped at this point and the microscopic crystals melted within 1 to 3 minutes. In one instance thawing was postponed for 13 minutes. Blood flow was invariably resumed briefly as soon as the crystals disappeared, but widespread stasis always developed secondarily within 1 to 7 minutes. This stasis was often reversible; the plugs of packed corpuscles were washed out by inflowing blood and normal flow was resumed. Partial to almost complete recovery occurred in every frozen mesentery except the one which had been kept in the solid state for 13 minutes.

b. *Effects of local cooling to between  $-2^{\circ}$  and  $+2^{\circ}\text{C}$ . on the relation between fluid movement and capillary blood pressure.* To form a suitable control series, 67 pairs of observations were made on 19 frogs (13 ♀, 6 ♂) at room temperatures between  $22.5^{\circ}$  and  $25.5^{\circ}\text{C}$ . The results are charted in figure 1 (left). In agreement with earlier observations (7), the rate and direction of fluid movement varied directly with capillary blood pressure. The correlation coefficient of  $+0.64$  and "t" value of 6.72 indicate a highly significant relationship between the 2 variables. Regression lines and their equations, calculated from the data, are shown in the figure.

Points representing 39 similar pairs of measurements on the cooled mesenteries of 7 frogs (5 ♀, 2 ♂) are shown in figure 1 (right). The temperature of the chamber ranged from  $-3^{\circ}$  to  $-7^{\circ}\text{C}$ . (mesenteries approximately  $-2^{\circ}$  to  $+2^{\circ}\text{C}$ .). Transient variations of temperature beyond these limits were recorded in 5 instances. The individual points are more scattered than in the control series because the conditions under which these observations were made were unavoidably less constant. The temperature of an individual capillary wall and its contained blood depended not only on the temperature of the chamber below the capillary and that of the air above, but also on the rate of blood flow. Rapid or slow entry of arterial blood at or near room temperature into the chilled capillary under observation would respectively increase or decrease the difference between the temperature of the chamber and the temperature of the capillary wall and its contained blood up to the moment when blood flow was obstructed, and for at least a few seconds thereafter. Nevertheless, the correlation coefficient of  $+0.39$  and "t" value of 2.58 indicate, for this series also, a

significant relationship between capillary blood pressure and the rate of fluid movement.

Comparison of the general distribution of points in the two charts of figure 1 shows that local cooling *a*, decreased the rate of filtration even at relatively high capillary pressures, and *b*, increased the general tendency toward absorption. Thus, filtration rates above  $0.01 \mu^3/\mu^2/\text{sec.}$  were rare in the cooled mesenteries, even when capillary pressure was high, although they occurred frequently at  $22.5^\circ$  to  $25.5^\circ\text{C.}$  At  $-2^\circ$  to  $+2^\circ\text{C.}$ , absorption appeared as often as filtration

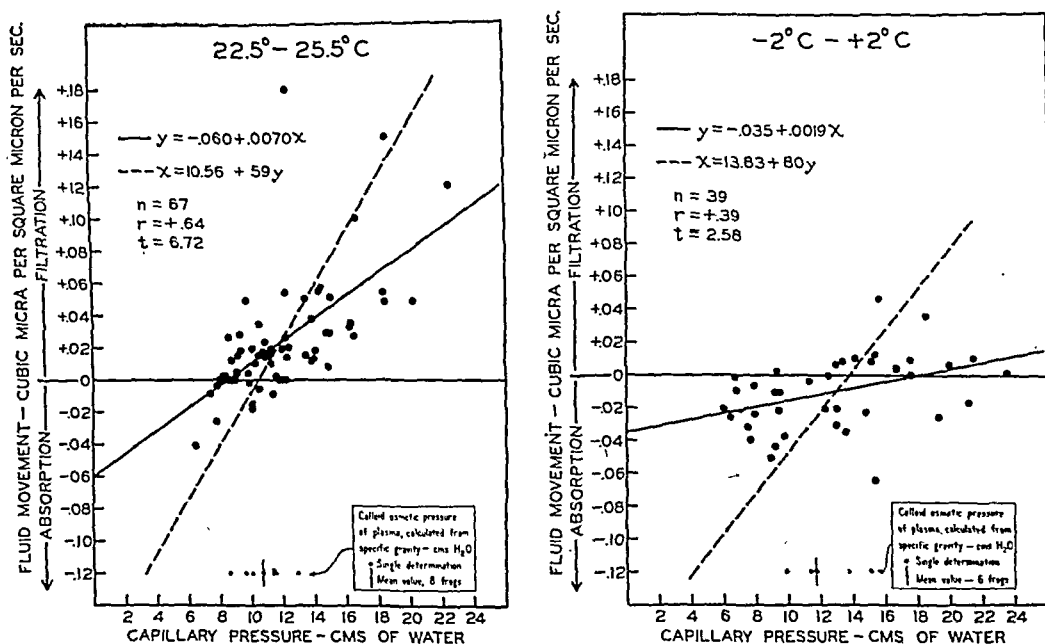


Fig. 1. (left) Chart showing relation between capillary blood pressure and fluid movement through walls of single mesenteric capillaries of the frog at room temperature ( $22.5^\circ$  to  $25.5^\circ\text{C.}$ ) Experimental points derived from 19 mesenteries; calculated colloid osmotic pressures (shown in lower part of figure) from 8 of these frogs.

(right) Chart showing the effect of local cooling of mesentery to between  $-2^\circ$  and  $+2^\circ\text{C.}$  Experimental points from 7 mesenteries; calculated colloid osmotic pressures from 6 of these frogs.

when capillary pressure was above 12 cm. of water, but at room temperature absorption never occurred at pressures above this level.

These changes may be expressed more quantitatively by comparing the regression lines whose equations have been calculated from the two sets of data, as summarized in figure 2. For the cooled mesentery (lower left) both regression lines are rotated clockwise and displaced to the right with respect to the lines fitting the control data (upper left, fig. 2), suggesting *a*, that the lessened filtration at all pressures is due to a decrease in capillary permeability, and *b*, that the greater absorption is due to an increased effective osmotic pressure of the blood.

For each set of observations the regression line having an equation of the general form  $y = a + bx$  expresses the experimental conditions most appropriately. Of the two variables,  $x$ , or capillary blood pressure, can properly be regarded as the independent variable because it was measured with the greater accuracy and is generally conceded to be one of the prime factors controlling  $y$ , the rate of fluid movement and the dependent variable. The slope of this line,  $b$ , is the "filtration constant" of the membrane because it indicates the volume of fluid passing through unit area of membrane in unit time per unit difference in capillary pressure.

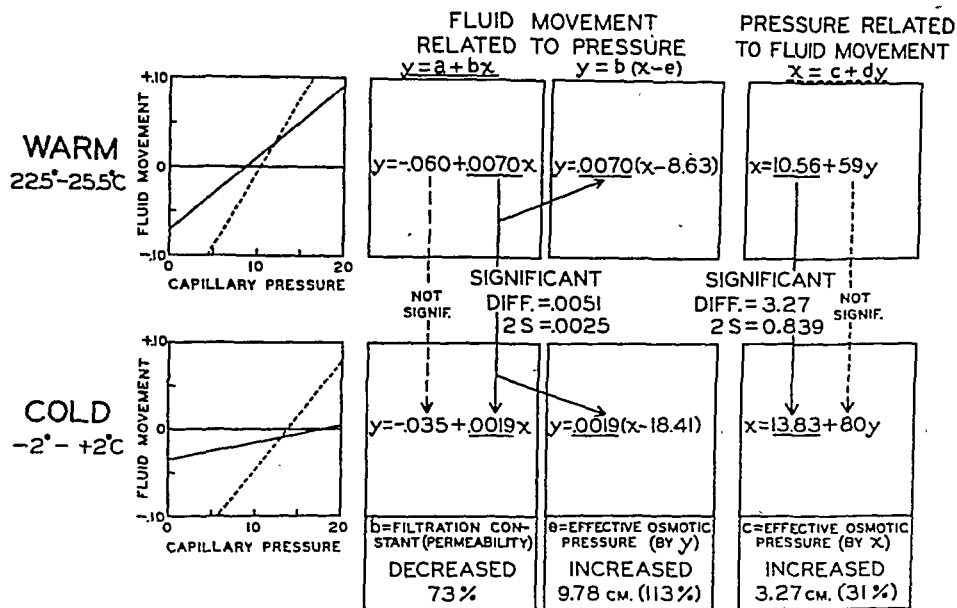


Fig. 2. Comparison of the regression lines expressing fluid movement as a function of capillary blood pressure ( $y = a + bx$ ) and capillary blood pressure as a function of fluid movement ( $x = c + dy$ ) in mesenteries at 22.5° to 25.5°C. and -2° to +2°C. Twice the standard deviations of the differences are shown in middle of figure; percentile differences are shown in lower portion of figure.

Under control conditions (22.5° to 25.5°C.) the filtration constant was 0.0070 which agrees quite closely with the constants observed by Landis (7, 11) in several studies of the frog's mesenteric capillaries at room temperature<sup>3</sup>. The filtration constant obtained during local cooling of the mesentery to between -2° and +2°C. was only 0.0019. This 73 per cent decrease in the constant is statistically significant (12) as shown under the  $y = a + bx$  equation in figure 2.

<sup>3</sup> The filtration constants originally reported (7, 11) ranged from 0.0056 to 0.0080, but these values were calculated from the  $x = c + dy$  regression lines and are slightly higher than those calculated from the  $y = a + bx$  lines fitting the same data. These constants have therefore been recalculated on the latter basis and found to range between 0.0048 and 0.0074. The differences between values obtained from the earlier data by the two equations are relatively small because the spread of individual points was less and the correlation coefficients were higher than in the observations reported here.

c. *Effective osmotic pressure of capillary blood during cooling.* The forces ordinarily opposing capillary blood pressure are  $a$ , the effective osmotic pressure of the blood, and  $b$ , tissue pressure. The latter appears to be negligible in the exposed mesentery of the frog. Hence the effective osmotic pressure of the blood is indicated by the intercept of the regression line with the  $x$ -axis, since this is the point of zero fluid movement. By re-arrangement of the equation  $y = a + bx$  to the form,  $y = b(x - c)$ , values for the intercepts,  $c$ , are obtained which indicate that the blood had an effective osmotic pressure of 8.6 cm. water in the control mesenteries and 18.4 cm. water in the cooled mesenteries, an increase of 9.8 cm. water or 113 per cent. It would be unwise, however, to attribute more than qualitative meaning to this figure because the constant  $c$  is derived in part from the constant  $a$  and the differences between values of  $a$  for the two series are not significant (see fig. 2).

The other regression lines (dotted) having equations of the form  $x = c + dy$  and therefore expressing capillary blood pressure in terms of fluid movement are more appropriately used for determination of pressure at the point of zero fluid movement. The constant,  $c$ , or intercept, indicates the effective osmotic pressure of the blood directly and in a form which permits a valid test of significance (12). In the control experiments, the effective osmotic pressure of the blood determined in this way was 10.5 cm. water, again in fair agreement with the range of 11.5 to 11.7 observed earlier by Landis for mesenteric capillaries at room temperature (7, 11). In the cooled mesenteries the effective osmotic pressure of the blood was 13.8 cm. water as shown by the corresponding intercept. The increase of 3.3 cm. water or 31 per cent associated with cooling to between  $-2^\circ$  and  $+2^\circ\text{C}$ . is significant (12) as shown under equation  $x = c + dy$  in figure 2. Conversely the difference between the slopes,  $d$ , calculated by this equation, is not significant.

d. *Capillary diameter and capillary blood pressure during cooling.* True capillaries were used exclusively for these studies and were selected according to anatomic criteria, viz., by their thin walls and typical relation to arterioles and venules. "Direct channels" or arterio-venous communications were excluded because their structure and behavior differ from those of true capillaries (13, 14). The diameters of the capillaries studied were almost identical in the two series. In the control mesenteries they ranged from 13 to 30  $\mu$ , averaging 20  $\mu$ , while in the cooled series they ranged from 15 to 30  $\mu$  and also averaged 20  $\mu$ . The distribution of capillary pressures is shown in figure 3. Except for the slightly greater frequency of pressures below 8 cm. water in the cooled mesenteries, there were no striking differences.

e. *Plasma protein concentration and colloid osmotic pressure.* Table 1 shows the specific gravities of plasmas and of ultrafiltrates, together with the calculated plasma protein concentrations of blood from 8 frogs of the control series and from 6 whose mesenteries had been cooled. Protein concentrations were multiplied by a factor of 4 to convert to equivalent colloid osmotic pressures (15). These ranged from 8.6 to 12.9 cm. water with an average of 10.7 cm. water for the controls, and from 9.9 to 15.1 cm. water with an average of 11.9 cm. water for

the animals with cooled mesenteries. The calculated colloid osmotic pressures are shown also in the lower portions of the two charts of figure 1. The averages in figure 1 have been weighted to allow for the number of experimental points represented by each specimen of plasma.

f. *Variations in concentration of plasma proteins in winter and summer frogs.* The control observations were begun initially during December, 1944, using winter frogs which were kept in an ice refrigerator and partly immersed in water until just before use. Absorption of fluid by the mesenteric capillaries was never observed in these animals, even at the lowest capillary blood pressures obtainable. The specific gravity of plasma from 4 specimens was therefore measured first by an approximate method (table 2, line 1) in order to determine whether this unusual result was due *a*, to an increase in capillary permeability

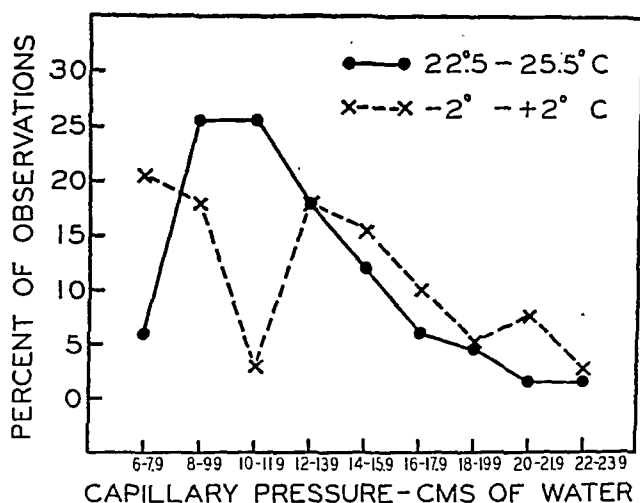


Fig. 3. The distribution of capillary blood pressures observed in 67 determinations in mesenteries at 22.5° to 25.5°C. and in 39 determinations in mesenteries between -2° and +2°C.

with lowered effective colloid osmotic pressure, caused by leakage of protein through the capillary wall, or *b*, to an absolute reduction in colloid osmotic pressure caused by hypoproteinemia, without any increase in capillary permeability. The latter explanation appears the more likely for two reasons. *a*. In 4 frogs kept in an identical fashion partly immersed in water and in a refrigerator, the usual absorption of fluid at low capillary pressures was observed after injecting 1 or 2 cc. of 25 per cent bovine albumin solution into the lymph sac. As shown in table 2, line 2, the average total specific gravity of plasma from these injected animals was 1.022. This can be compared with an average of 1.017 (line 1) for plasmas of animals whose capillaries failed to absorb. The difference is equivalent to about 1.7 grams of protein per 100 cc. of plasma. *b*. Specific gravities of plasmas and of the corresponding ultrafiltrates were then measured more accurately in a group of 9 winter frogs, also kept partly immersed in water in an ice box. In these the average plasma protein con-

centration was 1.8 per cent (table 2, line 3), which is considerably lower than the 2.7 to 3.0 per cent found in summer frogs kept under dripping water at about 22° C. (table 1).

If the plasma protein concentrations had not been estimated it might have been concluded erroneously that capillary permeability was increased in winter frogs. This calls attention again to the necessity of determining not only capil-

TABLE 1

*Specific gravities of plasma and ultrafiltrate of arterial blood, with the plasma protein concentrations and colloid osmotic pressures calculated from them*

(Summer frogs, July and August)

	SPECIFIC GRAVITY		CALCULATED PROTEIN CONC.	CALCULATED COLLOID OSMOTIC PRESSURE	NUMBER OF EX- PERIMENTAL POINTS PER FROG
	Plasma	Ultrafiltrate of plasma	gm./100 cc.	cm. water	
Mesenteries at room temperature 22.5° to 25.5° C.	1.0140	1.0056	2.9	11.5	6
	1.0129	1.0056	2.5	10.0	4
	1.0146	1.0063	2.9	11.4	5
	1.0123	1.0060	2.2	8.6	6
	1.0138	1.0067	2.4	9.7	3
	1.0138	1.0058	2.9	11.4	6
	1.0157	1.0063	3.2	12.9	3
	1.0161	1.0083	2.7	10.7	5
Average . . . . .	1.0141	1.0063	2.7	10.7	
Weighted average per experimental point..				10.8	
Mesenteries at -2° to +2° C.	1.0170	1.0070	3.4	13.7	4
	1.0143	1.0071	2.5	9.9	6
	1.0164	1.0054	3.8	15.1	5
	1.0154	1.0076	2.7	10.7	7
	1.0139	1.0060	2.7	10.8	5
	1.0152	1.0069	2.8	11.4	7
Average . . . . .	1.0154	1.0067	3.0	11.9	
Weighted average per experimental point..				11.7	

lary blood pressure but also the existing concentration of plasma proteins before attributing anomalies of fluid movement to changes in capillary permeability per se.

Variability of the protein content of frog's blood has been noted by others (16, 17). In studying the movement of fluid through the walls of single capillaries by the same methods which have been described here, Wind (18) encountered difficulties which he ascribed to variations between animals, although

his technique was probably responsible in large part for the lack of uniformity in results. Danielli (19) found in winter frogs that leakage of protein from the capillaries in perfused legs was greater than in summer frogs. It is not clear, however, whether the observed seasonal differences in the protein content of frog's blood are due to *a*, environmental temperature; *b*, nutritional hypoproteinemia; *c*, general hydration whenever frogs are almost totally immersed in water (20), or *d*, to true seasonal shifts in water balance (16).

In order to determine whether environmental temperature alone might be responsible for such differences between summer and winter frogs, two groups of 50 winter frogs were kept under dripping water in constant temperature rooms at 2° to 5°C. and at 30° to 32°C. respectively for 6 weeks. Blood was taken at weekly intervals from 2 to 4 animals in each group and pooled for determination of specific gravities of plasma and ultrafiltrate. As shown in table 2, lines 4 and 5, the plasma protein concentrations remained essentially the same in the 2 groups, from which it appears that environmental temperature was not the only factor responsible for the low plasma protein concentrations found in the series of winter frogs studied initially.

It has not so far been possible to keep the plasma protein concentrations of winter frogs uniform simply by controlling environmental temperature. However, if they are kept on netting under a constant, slow drip of tap water at room temperature for some days before use, their condition remains far better, and their plasma protein concentration higher as well as more constant.

DISCUSSION. Actual freezing injures the capillaries of man (3) and increases their permeability to protein. This is true also for the mesenteric capillaries of the frog, as shown by the transient capillary stasis and leakage which appear during the early moments of thawing. In both species freezing occurs only after some degree of supercooling, the deleterious effects of which appear to be due to mechanical disruption of the endothelium by ice crystals rather than to the effects of any specific temperature per se (4, 6).

At temperatures between 0° and 15°C. differences appear between the capillaries of man, a homoiothermic animal, and the frog, a poikilothermic animal which survives long exposure to low temperatures. In man increased permeability to fluid and protein can be detected at about 15°C. and becomes more pronounced as 0°C. is approached (1, 3, 4). This change has been attributed to certain histamine-like substances which arise from cellular injury (1, 3). In the frog, however, cooling the mesentery to between -2° and +2°C. decreased capillary permeability and increased the effective osmotic pressure of the blood. These effects are opposite to those produced by injury, e.g., by chemicals, which in previous studies (7) increased capillary permeability conspicuously and reduced, by leakage of protein, the effective colloid osmotic pressure of the blood. As might be expected from the frog's ability to survive during the winter, the capillaries of the frog are better adapted than those of man to withstand prolonged exposure to low temperatures, providing actual freezing is avoided.

In the frog's mesentery cooled to between -2° and +2°C. the filtration constant (fig. 2) was reduced by 73 per cent. For a given increment of capillary

pressure, fluid moved across the cooled capillary wall at a rate only one fourth that observed when the capillaries were at room temperature. This is not surprising, because the permeability constants of other natural membranes are changed in the same direction by change of temperature. In the frog, uptake

TABLE 2

*Specific gravities of plasma and ultrafiltrate of arterial blood and plasma protein concentrations calculated from them*

(Winter frogs, kept under various environmental conditions)

	DATES	NO. OF ANIMALS	CONDITIONS	SPECIFIC GRAVITY						CALCULATED PROTEIN CONC. GMS. PER 100 CC.		
				Plasma			ultrafiltrate			max.	min.	aver.
				max.	min.	aver.	max.	min.	aver.			
1.	Dec. 18-21	4	partly immersed, ice box	1.020*	1.013*	1.017*						
2.	Dec. 27-29	4	partly immersed, ice box. 1½-20 hrs. after albumin	1.023*	1.021*	1.022*						
3.	Feb. 28 to Apr. 22	9	partly immersed, ice box	1.0165	1.0095	1.0119	1.0081	1.0062	1.0069	3.1	1.2	1.8
4.	Jan. 19 to Mar. 5	9 pools of 2-4 animals each	2-5°C. constant temperature room, dripping water	1.0169	1.0101	1.0136	1.0074	1.0049	1.0059	4.1	1.5	2.6
5.	Jan. 19 to Mar. 5	8 pools of 2-4 animals each	30-32°C. constant temperature room, dripping water	1.0156	1.0092	1.0127	1.0082	1.0059	1.0071	4.2	0.8	2.1

\* Total specific gravity of plasma determined by copper sulfate method. Differences probably reliable, but this method was abandoned because absolute values were not reliable, and specific gravity of ultrafiltrate could not be measured.

of water through the skin increases four to fivefold when temperature rises 30°C. (20). In marine ova (21) and erythrocytes (22) the permeability constants of the surface membranes are related exponentially to temperature. The temperature coefficients of permeability may vary not only with species and the metabolic state of the cells (23) but also with the nature of the diffusing substance (24). While changes in the chemical, electrical or porous nature of a membrane may

be responsible for the effects of temperature on its permeability, it has also been found that temperature modifies the permeability constants of certain artificial membranes without changing their structures (25, 26). Therefore it is still precarious to assume that the observed decrease in the filtration constant of the chilled capillary wall in the frog is due to any simple change in the structure of the capillary endothelium per se.

The increase of at least 31 per cent in effective osmotic pressure of the blood when the mesenteric capillaries were cooled to between  $-2^{\circ}$  and  $+2^{\circ}\text{C}$ . is more unique and more difficult to explain. At least four possibilities must be considered. 1. The *absolute colloid* osmotic pressure of the whole circulating plasma might have been increased by local cooling of the mesentery. 2. If the capillary wall had been originally somewhat permeable to protein, it might conceivably have become less permeable to protein when cooled, and then the *effective colloid* osmotic pressure of the blood within the capillaries would have risen. 3. The capillary wall, ordinarily impermeable to protein but highly permeable to electrolytes, glucose, urea, amino acids and other non-protein constituents of plasma at room temperature, might have become relatively less permeable to these substances when cooled; the increase then would concern the *non-protein effective* osmotic pressure. 4. Blood entered the cooled capillaries at a temperature higher than that of the tissue fluid surrounding the vessels. The resulting temperature gradient from capillary blood to tissue fluid might have produced a difference between the total osmotic pressure of these two fluids and thereby influenced fluid movement. This *thermosmotic* increase in *total effective* osmotic pressure might modify the action of the well known forces included in the Starling hypothesis.

The first possibility can be discarded at once because the protein content of the circulating plasma was practically the same in the control animals and in those whose mesenteries had been cooled. The second possibility can also be discarded. It requires the assumption that the capillary wall is normally somewhat permeable to protein molecules at room temperature. Although the cutaneous capillaries of the frog are known to be highly permeable to protein (17, 27) this is apparently not true of the frog's mesenteric capillaries according to previous (7) and present studies. In figure 1, the band of experimental points crosses the abscissa, or the line of zero fluid movement, at capillary pressures which correspond almost exactly to the colloid osmotic pressures calculated from the specific gravities of plasma and ultrafiltrate. Therefore it is unlikely that the capillary filtrate at  $22.5^{\circ}$  to  $25.5^{\circ}\text{C}$ . contained significant amounts of protein. It follows that the increase of 31 per cent in the net force opposing filtration at  $-2^{\circ}$  to  $+2^{\circ}\text{C}$ . could not have been due to an increase in effective colloid osmotic pressure.

Third, the possibility still exists that the capillary membrane, made less permeable by the cold, might thereby impede the movement of smaller molecules which pass freely through the warmer capillary wall. If the average size of the endothelial pores were decreased sufficiently to restrict filtration of amino acids, glucose, urea and electrolytes more than the filtration of water, then *non-protein*

osmotic pressure within the capillary would rise slightly and temporarily. Hence the rate of movement of water would be temporarily less for any given difference between capillary pressure and colloid osmotic pressure. It has been estimated that the apparent size of the pores in the capillary wall varies between about  $7\text{\AA}$ . and  $38\text{\AA}$ ., with the majority between  $10\text{\AA}$ . and  $15\text{\AA}$ . (28, 29). Since the equatorial diameters of glucose, urea, amino acids and even some electrolytes with their enveloping water mantles (30) measure  $7\text{\AA}$ . or more, it is possible that a significant reduction in the average pore size of the membrane might interfere with the filtration of these molecules. Such an explanation must remain entirely hypothetical at present, because estimates of pore size are based on indirect evidence only and even the existence of pre-formed pores has not been proved.

Fourth, movement of fluid by thermosmosis might well occur when relatively warm blood flows continuously through capillaries surrounded by cooler tissue fluid. If blood were separated from tissue fluid by a membrane permeable only to water and the temperature difference between them were only  $1^{\circ}\text{C}$ ., a temporary osmotic pressure difference of 200 cm. water might develop between these two fluids. Rapid diffusion toward equilibrium would, of course, greatly diminish any such difference in osmotic pressure because the capillary wall, even when cooled, is permeable to electrolytes. The increase of 3.3 cm. water in effective osmotic pressure within the capillaries of the cooled mesenteries might thus be explained as the result of a thermosmotic difference in total osmotic pressure which was simultaneously being reduced by rapid diffusion. Somewhat analogous results have been reported for *in vitro* experiments (31) in which suitable animal membranes were used. The flow of fluid was from the cool to the warm chamber, but the maximum difference in osmotic pressure caused by a large temperature difference ( $80^{\circ}\text{C}$ .) was only 3.83 cm. water. These studies have shown that the rate of thermal diffusion and even its direction are affected by the nature of the membrane separating the phases. Predictions based on purely theoretical grounds are precarious because there is some evidence (32) that differences in vapor pressure may cause thermal diffusion to develop an osmotic pressure opposite to that predicted on the basis of the kinetic theory. Unfortunately the models used for *in vitro* studies of thermosmosis have not simulated conditions in the living animal where warm capillary blood is being renewed continually. The results which have been reported are difficult to interpret because equilibrium conditions were not approached and temperature differences were not held constant (32, 33).

Thus, in view of the available evidence, this increase of effective osmotic pressure cannot be ascribed to changes in concentration of the plasma protein in circulating blood or to changes in effective colloid osmotic pressure. It is not possible at present to choose between the two remaining factors,—*a*, decreased permeability to non-colloidal constituents of blood, and *b*, thermosmosis. In either case an addition to the Starling hypothesis seems necessary to explain fully the effect of cold on the movement of fluid through the capillary wall of the frog. Experiments more appropriate to the special problem are in

progress because thermosmosis may conceivably aid in explaining certain anomalies of fluid movement through the capillary wall which have been difficult to understand on the basis of known forces.

#### SUMMARY

1. During local cooling of the frog's mesentery, no signs of capillary injury or of leakage of protein from the capillaries appeared until after the tissue had been frozen. Capillary stasis invariably developed as soon as blood flow was resumed after thawing but this stasis was often reversible, particularly after very brief periods of freezing.

2. Micromanipulative studies of single capillaries in the frog's mesentery indicated that local reduction of tissue temperature to between  $-2^{\circ}$  and  $+2^{\circ}\text{C}$ . (slightly above the freezing point) decreased the filtration constant of the capillary wall by 73 per cent. Under similar circumstances, the effective osmotic pressure of the blood within the capillaries increased by at least 31 per cent.

3. Possible reasons for this increase in effective osmotic pressure have been discussed. The change cannot be attributed to an increase in colloid osmotic pressure, either absolute or effective. On theoretical grounds it might be explained by *a*, decreased permeability of the capillary wall to certain non-protein constituents of the plasma, or *b*, by thermosmosis. It is impossible at the present time to state which of these two factors is the more important.

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# THE EFFECT OF BLOOD CELL CONCENTRATION ON THE TRANSMISSION RATE OF THE ARTERIAL PULSE<sup>1</sup>

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In attempting to analyze changes in the rate of pulse transmission that have been observed at various stages of hemorrhagic hypotension and circulatory shock the question arose as to whether these rates might be influenced by the hemodilution and subsequent hemoconcentration known to occur under these conditions (5). The answer to this question is not to be found in previous formulations of the factors determining the rate of pulse transmission (1, 3), for they consider blood as a simple homogeneous fluid which influences transmission rate only by virtue of its density; its apparent viscosity—a factor primarily determined by the relative concentration of blood cells to plasma—is not taken into consideration. A series of experiments was therefore designed to test this relationship. When experiments on three dogs proved uniformly negative the study was discontinued. However, since the experiments were carried out under well controlled conditions the results appear to warrant a brief report.

To assure adequate transmission distances, large dogs were used and simultaneous pressure pulses were recorded from the origin of the carotid artery and from the dorsalis pedis artery. The arterial cannulae were connected to optical manometers having natural frequencies of at least 100/sec., with a sensitivity of 1 mm. deflection for every 2 mm. change in pressure. The pulses were recorded on photographic paper traveling at a speed of 120 mm. per second. Since comparable transmission rates can only be expected with equivalent diastolic pressures (1), it was necessary to stabilize the arterial pressure at some arbitrary level that could be kept relatively uniform for all recordings. As an experimental expedient the level selected was a mean pressure about 30 mm. below the initial blood pressure and this pressure was obtained before each recording by bleeding the animal until the pressure became stabilized at this level. The presence of some respiratory variation in the pressure values of successive pulses made it possible to select pulses from each of the recordings that had approximately the same diastolic pressure.

The dogs were anesthetized with barbitol sodium (280 mgm./kg.IV.) and heparinized. During the preliminary operative procedures the animals were subjected to a series of bleedings until about 600 cc. of blood had been withdrawn, care being taken to avoid lowering the blood pressure to critical levels. This blood was centrifuged, the plasma set aside, and the cells reinjected so as to produce an initial hemoconcentration. After obtaining an initial recording of the pressure pulses and a blood sample for hematocrit determination of the animal in this hemoconcentrated condition, progressive hemodilution was ac-

<sup>1</sup> This investigation sponsored by a grant from the Commonwealth Fund.

complished by 100 cc. infusions of the dog's own plasma recovered from the initial bleedings and from the subsequent stabilization bleedings. Towards the latter part of the experiments it was necessary to dilute this plasma with 20 to 30 cc. of saline in order to make up the 100 cc. volume. Simultaneous pressure pulse recordings and hematocrit determinations were made at least five minutes after each plasma infusion to allow time for adequate mixing.

At the conclusion of an experiment transmission distances were determined by measuring the length of a flexible wire that had been passed through the lumina of the appropriate blood vessels. It was then possible to convert the data obtained from the optical recordings to transmission rates of the pulse from the arch of the aorta to the point of cannulation of the dorsalis pedis artery.

TABLE 1

HEMATOCRIT PER CENT	TRANS. RATE METERS/SEC.	HEART RATE BEATS/MIN.	CENTRAL SYSTOLIC P.	CENTRAL DIASTOLIC P.
Dog 1—21 Kgm. male; transmission distance: 69.0 cm.				
64	7.6	236	124	103
56	7.6	227	123	104
47	7.7	207	123	104
42	7.7	200	126	106
35	7.6	208	125	104
30	7.7	220	123	104
Dog 2—15 Kgm. male; transmission distance: 63.9 cm.				
61	6.8	112	117	90
50	6.8	110	118	90
42	6.9	133	118	91
37	6.8	130	118	90
31	6.9	115	120	90
27	7.5	180	116	90
22	7.8	204	118	89

Two of the experiments are summarized in table 1. These data have been condensed by tabulating the values for every alternate record so that successive values represent the hemodiluting effect of 200 cc. of plasma. Heart rates and pressure values of the central pulse are also given to indicate the degree of constancy of circulatory conditions. It will be observed that in the case of the first animal there was no significant change in transmission rate of the pulse over the entire range of hematocrit values studied. This animal always exhibited a marked tachycardia when the blood pressure was lowered to the recording level of 115 mm. mean pressure, suggesting a fairly regular excitation of cardiovascular compensatory mechanisms in each instance. In the second animal it was possible to stabilize the blood pressure at the recording level of 100 mm. mean arterial pressure without eliciting any significant tachycardia or other signs of cardiovascular compensation as long as hematocrit values were above 30 per cent. Under these conditions there was also an essentially constant

transmission rate. However, when the hematocrit of this animal was dropped below 30 per cent and the pressure lowered to 100 mm. evidence of compensatory activity appeared in the marked acceleration of heart rate and accompanying this was a significant increase in transmission rate. It would appear that this acceleration in transmission rate indicated in the last two values of table 1 was not a direct consequence of the hemodilution but rather a result of the compensatory vasoconstriction that may be assumed to have been occurring under these conditions.

The rate of pulse transmission varies inversely with the square root of the density of the blood (1). On the basis of a specific gravity for whole blood of 1.057 and for plasma of 1.025 (2), we may estimate the maximum change in blood density produced here as being something less than 3 per cent, which should produce a change in pulse transmission rate of the order of 1.5 per cent. A change of this order of magnitude is obviously within the experimental error of the measurement of transmission rate. More significant, however, is the effect of hematocrit values on blood viscosity which on the basis of the data of Whittaker and Winton (4) may be estimated as a change in the apparent viscosity *in vivo* of about 50 per cent over the range of hematocrits studied here. The data demonstrate, therefore, that a change in apparent blood viscosity of the order of 50 per cent has no significant effect on the rate of transmission of the arterial pulse.

#### SUMMARY

When the cell concentration of the blood of dogs is reduced from hematocrit values of 60 per cent to values of 30 per cent there is no significant change in the rate of transmission of the arterial pulse from the arch of the aorta to the dorsalis pedis artery. This is interpreted as indicating that the factor of blood viscosity may be neglected in formulations of pulse kinetics.

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# CHRONIC MODERATE HYPERVITAMINOSIS D IN YOUNG DOGS<sup>1</sup>

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Much experimental work beginning with the observations of Kreitmaier and Moll (1) has demonstrated that excessive amounts of vitamin D produce toxic effects. Some of the questions which have been incompletely answered concern the relative toxicity of the various forms of the vitamin, the protective value of other nutrients, particularly other vitamins, and the reversibility of the tissue changes produced by the hypervitaminosis. In view of the recent introduction of massive dose therapy for the prevention and cure of rickets further light on these problems appears desirable and necessary.

This study was undertaken to obtain new information on these questions and in the hope of unravelling further the normal mode of action of vitamin D through observation of its effects when administered in excess of the usual dosage and along with a diet of the calcium content characteristic of infant diets.

The study is reported in some detail because it included control of several factors not previously controlled in similar experiments. The dogs used were purebred cocker spaniels of uniform origin and produced under exact control of diet and care of their mothers during pregnancy and lactation. They were kept on the same purified diet throughout the experiment which extended over ten months. Since they were kept in separate metabolism cages and never left the laboratory except for occasional exercise on an adjacent concrete runway, they were not exposed to infections or parasites.

The conditions which affect the kind and severity of hypervitaminotic symptoms have been well reviewed by Reed, Struck and Steck (2). The importance of these seems to be in the following order: (a) the amount of excess and the period of administration, (b) the character of the diet, particularly its Ca and P content, (c) the age and size of the subject, (d) the efficiency of the renal apparatus, (e) the species. In addition, some evidence has also been found, (f) that the vitamin A status of the subject may affect the toxicity (3, 4), and (g) that the form of vitamin D used (5) may control the speed of appearance and disappearance of the toxic changes. These latter 2 factors were the chief variables examined in the study here reported.

**EXPERIMENTAL PROCEDURE.** Purebred cocker spaniels, born and reared in the laboratory colony, were used as subjects. When five to eight weeks of age, these dogs were placed on the experimental diet made up of casein 45.8, sucrose 20.9, cornstarch 19.4, hydrogenated cottonseed oil 10.0, salts no. 5<sup>2</sup> 2.4,

<sup>1</sup> This study was supported in part by a grant from the Nutrition Foundation, Inc.

<sup>2</sup> Salt Mix no. 5 had the following composition:

KH <sub>2</sub> PO <sub>4</sub> .....	20.3	Fe citrate.....	2.2
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> .....	56.3	K I.....	0.005
MgSO <sub>4</sub> .....	4.9	Na F.....	0.068
NaCl.....	22.4	MnSO.....	0.021
Ca lactate.....	98.7	K Al (SO <sub>4</sub> ) <sub>2</sub> .....	0.006

$\text{CaCO}_3$  1.5. As source of the B vitamins, 1 gram dry brewery yeast and 1 gram wheat germ per kgm. per day were given separately. Three litters, 13 dogs in all, were placed on this diet at weaning. Previous experience with this diet supplemented with vitamins A and D, had shown that on it young dogs grew well and that reproduction was successful. The diet contained 0.9 to 1.0 per cent calcium and 0.7 to 0.8 per cent phosphorus and this composition was maintained consistently throughout the experiment. The dogs were kept in separate metabolism cages. In the first three or four months the dogs consumed about 50 grams per kgm. per day of the basal diet, but this amount later decreased to about 20, as the weight curves reached a plateau. The calcium and phosphorus intakes per kilogram per day were therefore approximately 0.48 and 0.37 gram at first, decreasing to 0.19 and 0.15.

This concentration of calcium and phosphorus was chosen since it represented a fair approximation to that of milk solids, the usual diet of the infants treated by single or repeated massive doses of vitamin D.

The vitamin D sources were irradiated ergosterol,<sup>3</sup> halibut liver oil<sup>4</sup>, tuna liver oil<sup>5</sup>, and delsterol<sup>6</sup>, and the vitamin A sources, the same fish liver oils, shark liver oil<sup>7</sup>, and crystalline carotene<sup>8</sup>. Since the halibut liver oil had a high A:D ratio, 13 to 1 or 11 to 1, and the tuna liver oil a low ratio, 0.8 to 1, the former was used chiefly as source of vitamin A and the latter only for the group given excessive amounts of both vitamins. The arrangement of the experimental groups is shown in table 1.

The vitamin doses were made up of the calculated amounts of liver oils, irradiated ergosterol in oil, delsterol or carotene, diluted with cottonseed oil to an appropriate volume and fed to the dogs by capsule daily. For example, the dose for one group was made up of 1.1 gram halibut liver oil (202 I. U. vitamin A and 61 I.U. vitamin D per mgm.) diluted to 300 grams with cottonseed oil and fed daily in the amount of 1 gram per kgm. to dogs 1 and 5, to provide 800 I.U. vitamin A and 72 I.U. vitamin D. Each of the liver oils, the irradiated ergosterol solution and the delsterol were examined by biological assay for vitamin D and the liver oils by both biological and the Carr-Price chemical method for vitamin A.

For convenience, the vitamin D of irradiated ergosterol is designated as vita-

<sup>3</sup> The irradiated ergosterol in oil was supplied by Mead, Johnson and Company of Evansville, Indiana. It contained 800,000 I.U. vitamin D per gram, no toxisterols, but presumably some tachysterol.

<sup>4</sup> The halibut liver oil was supplied by E.R. Squibb and Sons of New York and consisted of two lots, one with 202,000 I.U. vitamin A and 1,430 I.U. vitamin D per gram and the other with 160,000 I.U. vitamin A and 1,360 I.U. vitamin D per gram.

<sup>5</sup> The tuna liver oil, supplied by Mead, Johnson and Company had 47,000 I.U. vitamin A and 61,000 I.U. vitamin D per gram.

<sup>6</sup> The delsterol which contained 300,000 I.U. vitamin D per gram was supplied by Dr. James Waddell of E. I. duPont de Nemours and Company, New Brunswick, N. J.

<sup>7</sup> The shark liver oil, supplied by Mr. T. Sanford of F. E. Booth Company, Berkeley, California, contained 130,000 I.U. vitamin A and 85 I.U. vitamin D per gram.

<sup>8</sup> Crystalline carotene, 90 per cent beta and 10 per cent alpha, supplied by General Biochemicals, Inc., Chagrin Falls, Ohio.

min D<sub>2</sub> and that of fish liver oils and irradiated animal sterols (delsterol) as vitamin D<sub>3</sub>. There is no certainty, however, that all the vitamin D present in tuna liver oil and halibut liver oil is identical with irradiated 7-dehydrocholesterol, designated vitamin D<sub>3</sub>, and presumably the potent substance of delsterol.

TABLE 1  
*Amounts and sources of vitamins D and A fed young dogs*

GROUP	DOG	VITAMIN D PER KG. PER DAY	VITAMIN A PER KG. PER DAY	TOTAL PERIOD ON DIET	NUMBER OF EXCESS DAILY DOSES GIVEN	TOTAL VITAMIN D GIVEN
		<i>I. U.</i>	<i>I. U.</i>	<i>days</i>		<i>I. U. × 1000</i>
1. Optimum vitamin D and vitamin A	1♂	72, tuna liver oil	800, tuna liver oil	302		161
	5♂	oil	and halibut liver oil	296		153
	9♀	72, irradiated ergosterol	800, halibut liver oil	296		119
2. Optimum vitamin D and excess vitamin A	2♂	72, halibut liver oil	10,000, halibut liver oil	302		161
	6♂			296		153
	8♀	72, irradiated ergosterol	10,000, carotene in oil	296		119
3. Excess vitamin D and excess vitamin A	11♀	10,000, tuna liver oil	10,000, tuna liver oil and halibut liver oil	296	236	15,500
	3♀	10,000, irradiated ergosterol	10,000, halibut liver oil	312	236	14,400
	7♂			296	236	18,900
	13♂*	10,000, irradiated ergosterol	10,000, shark liver oil	360	147 in 188 days	12,000
	12♂*	10,000, delsterol	10,000, shark liver oil	360	127 in 188 days	9,500
4. Excess vitamin D and optimum vitamin A	4♀	10,000, irradiated ergosterol	800, halibut liver oil	312	236	15,530
	10♀			296	236	12,080

\* These dogs were relieved of medication and allowed a recovery period of 146 days.

Serum calcium was determined at intervals, but blood inorganic phosphate was determined only once, just before the animals were sacrificed. The calcium method of Larson and Greenberg (6) and the phosphorus method of Fiske and Subbarow (7) were used.

At the end of the experiment all seven of the hypervitaminotic and two of the control dogs were killed and the soft tissues examined chemically and histo-

logically. The femurs, jaws and teeth were examined by roentgenograph and histologically. Vitamin A was determined chemically in the livers and kidneys and ascorbic acid in the adrenals.

Litter A, consisting of four dogs, nos. 1, 2, 3 and 4, was placed on the diet earlier than the others, at 39 days of age. From the 52nd to the 60th day of age dogs 3 and 4 were given daily 20,000 I. U. per kgm. vitamin D<sub>2</sub> (as irradiated ergosterol) and either 10,000 or 800 I.U. vitamin A per kgm. These animals immediately became ill, refused to eat, had diarrhea, vomited and lost weight. This dosage was obviously dangerously excessive, and since a chronic, rather than an acute hypervitaminosis was sought, the vitamin D was withdrawn. According to Steck, Deutsch, Reed and Struck (8) this level of vitamin D medication is safe for both adult dogs and human subjects, but on this diet and for these young dogs it was dangerously excessive. After 20 days of recovery during which the dogs improved rapidly, the dosing was resumed but at the level of 10,000 I.U. vitamin D<sub>2</sub> per kgm. per day.

One of the females of this litter, no. 3, was evidently a premature, since she was considerably smaller than the other members of the litter at birth, gained only 1.1 kgm. during the suckling period as compared with the litter average of 1.3 and lost 0.2 kgm. during the first two weeks on the diet. The other female, no. 4, subjected to the same treatment was able to maintain her weight during this period. The symptoms induced by the first doses of vitamin D were more severe in no. 3 than in any of the other animals and her recovery during rest periods was delayed and incomplete.

At this time litter B, consisting of seven dogs, nos. 5 to 11, which had been placed on the diet at 38 days of age, was 65 days old. Three of these animals, nos. 7, 10 and 11, were given 10,000 I.U. vitamin D per kgm. per day along with the two dogs from litter A. After six days of the dosing again there were serious signs of toxicity and after eight days the medication was discontinued. After 41 days' interval the vitamin D was again given at the 10,000 I.U. per kgm. per day level and was continued for 220 days. When this last period was begun the dogs were nearly four months old, and the normal males weighed 9 to 11 kgm., the females 8.0 kgm. The diarrhea disappeared in the experimental dogs, constipation appeared, the appetites were irregular, vomiting occurred occasionally, growth ceased, and in some cases stiff and painful gait developed.

Litter C consisted of two male dogs, nos. 12 and 13, weaned to the diet at 35 days of age. Administration of 10,000 I.U. vitamin D<sub>3</sub> per kgm. per day as delsterol to no. 12 and of vitamin D<sub>2</sub> as irradiated ergosterol to no. 13, was begun when they were 60 days old. Both dogs received 10,000 I.U. vitamin A per kgm. per day in shark liver oil. It was at once apparent that dog 12 was the more severely affected. After 18 days the medication was withdrawn and after 24 days in the case of no. 13 also. Thereafter in 164 to 170 days, dog 12 received the excess dosage on 109 days and dog 13 on 123 days. For 146 days, from the 8th to the 13th month of their age, they were free of the medication. The dog which had received delsterol made a more rapid functional recovery than did the dog which had received irradiated ergosterol. Gain in appetite, weight,

height and activity, and improvement in coat quality were all more striking in dog 12 than in dog 13.

*Growth.* The hypervitaminotic animals were stunted in both height and weight. Weight curves of litters A and B are shown in figure 1. In both litters the normal animals which received the excess vitamin A were larger at nearly all ages than those which received the smaller amount of vitamin A. This latter allowance, 800 I.U. per kgm. per day, is in excess of the amount usually considered adequate (9). The growth rate of the hypervitaminotic dog 11 given tuna liver oil, was also greater than that of the comparable animal 10 which was given the same amount of vitamin D but the lower amount of vitamin A, but dog 3, similarly comparable as to diet with dog 4, remained somewhat smaller throughout the latter part of the experiment than no. 4.

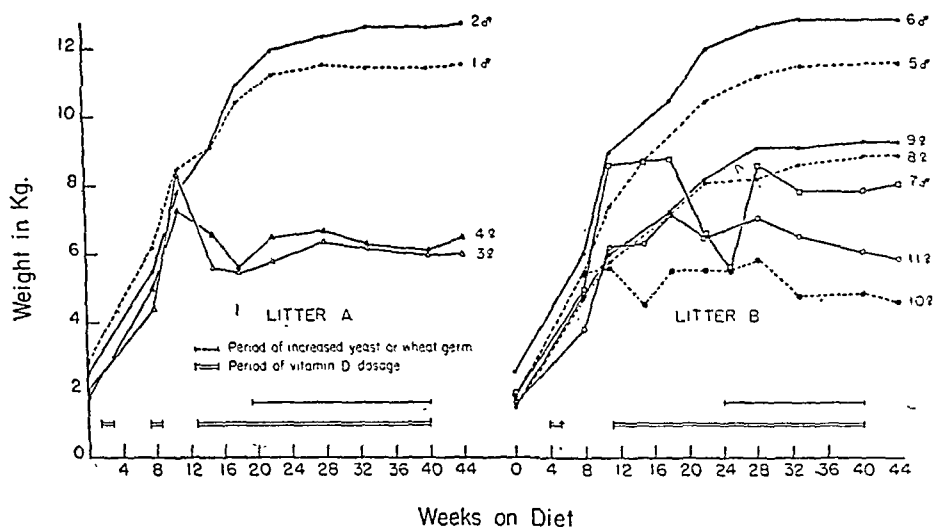


Fig. 1. Growth of young dogs as affected by excess vitamin D and optimum or excess vitamin A intakes. Dogs 1, 2, 5, 6, 8 and 9 were normal, 4 and 10 had excess vitamin D and optimum vitamin A, 3, 7 and 11 excess vitamin D and excess vitamin A.

*Serum calcium.* As was expected, the serum calcium level was raised in all the dogs given excess vitamin D (fig. 2). The highest levels were reached during periods of temporarily increased food intake and these periods were usually followed by abrupt loss of appetite, fall in body weight and reduced serum calcium. This occurred notably in the case of no. 7, but also in three other hypervitaminotic dogs of litters A and B when after the 24th week (litter B) or the 19th week (litter A) 4 grams per kgm. per day additional wheat germ or yeast was given them. This was done in the hope of improving the appetites which were poor in all the hypervitaminotic dogs. Wheat germ was given to nos. 7 and 4, yeast to nos. 3 and 10. There was an immediate increase in food intake and body weight in all four dogs. This continued for several weeks after which an abrupt fall in appetite and weight occurred with continued fluctuation in these factors thereafter. Since the calcium and phosphorus intake, except for the small amounts present in the wheat germ and yeast, was dependent

on the amount of food eaten, the rise in serum calcium following increased intake might be taken to be in response to the increased calcium ingested. But the high serum level thus stimulated in turn may have depressed other normal functions with resulting loss of weight, decreased intake and falling serum calcium level.

Under similar circumstances the dogs, nos. 3 and 7, given the excess vitamin D<sub>2</sub> as irradiated ergosterol maintained higher serum calcium levels than did no. 11 given vitamin D<sub>3</sub> in tuna liver oil.

Inorganic phosphorus, determined just before the dogs were sacrificed, was found to vary but little. There was no apparent relation between the P and

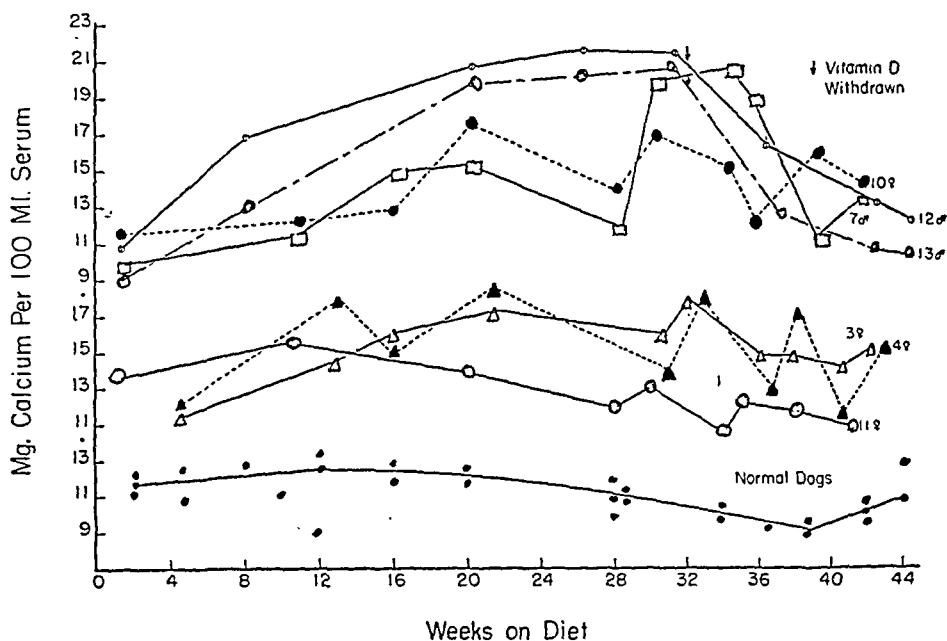


Fig. 2. Serum calcium of young dogs as affected by excess vitamin D and optimum or excess vitamin A intakes. Dogs 4 and 10 had optimum, the others excess, vitamin A intakes.

Ca serum levels. Control dog 6 had 8.7 mgm. per cent Ca and 4.5 mgm. per cent inorganic P, no. 11 had 11.5 and 6.0, no. 3, 15.5 and 5.2, no. 7, 13.9 and 4.1, no. 4, 15.4 and 4.7, no. 10, 13.8 and 4.4.

*Condition of tissues.* The two dogs given optimum amounts of vitamin D, nos. 1 and 6, at autopsy were found to be normal in all respects. The four controls which were not sacrificed were also in excellent condition and three were maintained in the colony as breeding stock for several years. The other male control, no. 5, which had been given the lower amount of vitamin A developed an infection after a few months and was sacrificed.

All of the hypervitaminotic animals presented abnormally calcified soft tissues in greater or less degrees.

The three dogs most affected by the treatment, nos. 3, 4 and 10, had extensive

calcium phosphate deposits in the heart, muscles, stomach wall, lungs and kidneys. Hard white stones were seen on the surface of the cardiac papillary muscles, the chordae tendinae were thick and hard, and bicuspid valves thickened and inelastic. Even the smaller blood vessels exhibited calcification. No stones were found in the kidneys but the renal tissue was tough and gritty, the cortico-medullary junction indefinite, streaked and mottled, the cortex reduced in width.

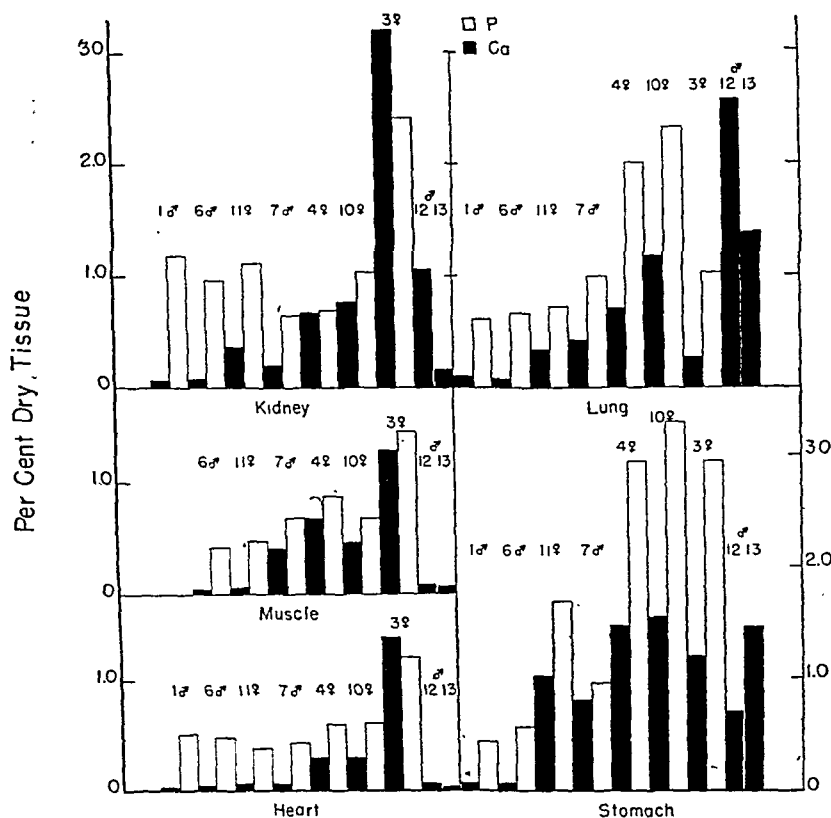


Fig. 3. Calcium and phosphorus content of soft tissues of young dogs as affected by excess vitamin D and optimum or excess vitamin A intakes. Dogs 1 and 6 were normal, 4 and 10 had optimum vitamin A and excess vitamin D, the others excess vitamin A and excess vitamin D.

Granular salt deposits were found adjacent to the bone extending along the tendons, in the muscle sheaths and connective tissue and laid down beside the ribs and between the vertebrae. These deposits looked as though a solution had seeped from the joints and deposited crystals in the adjoining tissues. Yet these bones were over-mineralized and the ash content of the femurs was somewhat greater, 62 per cent of dry extracted bone, than that of the normal dogs, 58 per cent. The long bones were extremely hard with irregular reduced marrow space, and greatly enlarged width of the shafts.

In dogs 7 and 11 calcareous deposits in the soft tissues were rare and the long

bones were more normal than in dogs 3, 4 and 10. Dog 11 which received tuna liver oil was most nearly normal of all.

*Jaws and teeth.* The normal control dogs 1 and 6 had large well-formed teeth, but the experimental dogs had small, poorly placed teeth with deformities of the roots in some cases, pulp stones and some degree of malocclusion. These faults were most striking in 3, 4 and 10, less obvious in 7 and least in 11. The jaw bones were sclerotic and the cementum pathologically calcified. A full description of the dental conditions has been published (10).

Dogs 12 and 13 in spite of nearly five months' recovery period, had enamel hypoplasia, pulp stones, root deformities, severe osteoporosis, atrophy of alveolar crests, heavy tartar formation and typical paradentosis. The changes produced in jaws and teeth appeared to be irreversible (11).

*Kidney function in recovery.* Blood urea was determined in dogs 12 and 13 as an index of renal damage. The values were 13.0 and 10.5 mgm. per cent blood before the vitamin D was given and gradually increased during the dosing period to 56 and 34 at the time the vitamin administration was discontinued. At this time no. 12 exhibited severe albuminuria. After a month of recovery no. 12 had lower blood urea than no. 13 and at the end of the five months' recovery the value for the former was 14 and for the latter 15 mgm. per cent. The kidney of no. 12 exhibited degenerate and calcified glomeruli; necrotic debris in both proximal and distal tubules and some cloudy swelling. There were no calcium deposits in the glomeruli in the case of no. 13, but some degenerated glomeruli and casts and debris in some of the tubules. Thus, renal function appeared to be more rapidly regained by no. 12 but anatomical repair was less complete than in no. 13.

*Analysis of tissues.* Calcium and phosphorus were determined in the soft tissues of all the dogs sacrificed, as shown in figure 3.<sup>9</sup> The calcium content of kidney, lung, heart, stomach and femoral muscle of dogs 3, 4 and 10 was excessive. Kidney, lung and stomach of no. 12 and lung and stomach of no. 13 were likewise highly calcified. The latter two animals which had been allowed to recover from the medication for 146 days had nearly normal heart and muscle calcium. In these cases apparently the decalcification which may have occurred proceeded most rapidly in the muscle tissue and persisted longest in the lungs. Dogs 7 and 11 had decidedly less serious lesions than any of the other hypervitaminotic animals except in the stomachs which were nearly as much calcified as were those of 3, 4 and 10.

The proportion of excess phosphorus to excess calcium was in the neighborhood of 2 to 1 in the kidneys, hearts and femoral muscles of the three severely affected animals but was about 0.5 to 1 in the lungs and stomachs. The phosphorus of the tissues was not determined in the cases of dogs 12 and 13, in which calcium deposited in lungs and stomachs apparently persisted through the recovery period.

If the theory of Hofmeister (12) be accepted, that calcification occurs in alkaline tissues, then most serious and persistent deposits might be expected in

<sup>9</sup> Our thanks are due Nobuko Shimotori for many of these analyses.

gastric mucosa, bronchi and renal tubules since acid is secreted or excreted in these areas, leaving the cells relatively alkaline. The proportion of  $\text{CaCO}_3$  to  $\text{Ca}_3(\text{PO}_4)_2$  deposited in lungs and stomach mucosa of these dogs must have been greater than in the other tissues, as indicated by Ca:P ratios of these deposits. The deposits in skeletal muscle and heart had ratios close to that of tertiary calcium phosphate, indicating a possibly different mechanism of precipitation.

In the partially recovered animals, nos. 12 and 13, the Ca content of the lung was greater than in any of the acute cases, but that of hearts and muscles was normal, stomachs and kidneys intermediate. The removal of the accretions from areas of lower to higher alkalinity may be indicated.

The changes in calcium and phosphorus of the livers were not significant and were not included in figure 3. The normal dogs had 0.010 and 0.006 Ca per 100 gram liver solids, the recovered animals 0.004 and 0.007, and the 5 hypervitaminotic 0.010 to 0.027. The phosphorus was 0.90 and 0.74 per 100 grams liver solids in the 2 normal dogs and 0.61 to 0.76 in the 5 hypervitaminotic animals. Thus, slight increases in calcium and larger decreases in phosphorus were noted in the livers of the affected animals. There were no decreases in phosphorus of any of the other tissues of the severely hypervitaminotic animals as compared with the values found in the normal dogs.

The variable and slight calcification of tissues found by Reed, Dillman, Thacker and Klein (13) in hypervitaminotic dogs is not comparable with the data here presented since they used adult animals of varied origins, varied doses of viosterol and short periods of observation. Since their normal controls were likewise adults of varied history the variability of composition of their tissues was much greater than in the present study. Although the calcium and phosphorus content of the diet used by these investigators was not stated the calcium was noted in one chart as about 50 mgm. per kgm. per day (14). This is well below the approximate 300 mgm. Ca per kgm. received daily by the young dogs in the present study.

*Vitamin A deposition.* The vitamin A content of the livers and kidneys of 7 of the dogs was determined by the method of Davies (15). As shown in table 2 the dogs which received 800 I.U. vitamin A per kgm. per day had about one-tenth as much of the vitamin in the kidneys and only one-hundredth as much in the livers as did the 4 dogs which had received 10,000 I.U. vitamin A per kgm. per day. The 5 dogs which had received excessive amounts of vitamin D apparently stored about the same amounts of vitamin A as did the 2 normal control animals. The retentions in the 2 groups were in fact strikingly consistent. The dogs which received the smaller daily dose of vitamin A stored 3 to 8 per cent of the total intake and those which received the larger intake stored 36 to 44 per cent. This might be taken to indicate that the lower or "optimum" daily dose of 800 I.U. per kgm. was very little in excess of the actual needs of these young dogs.

*Adrenal ascorbic acid.* The three dogs which received the larger amount of vitamin A had smaller adrenal glands containing more ascorbic acid than did

three which received the smaller dose of vitamin A, regardless of vitamin D intake. Dogs 1, 10 and 4, of the latter group, had adrenals weighing 0.41, 0.55 and 0.64 gram and containing 0.16, 0.27 and 0.30 mgm. ascorbic acid. Dogs 7, 3 and 11, of the former group, had adrenals which weighed 0.30, 0.35 and 0.28 gram and containing 0.48, 0.53 and 0.45 mgm. ascorbic acid. The concentration of ascorbic acid in the glands of the latter group was about four times that of the former. The vitamin A of the adrenals was not determined. The possible antagonism of excess vitamin A or a toxic substance accompanying vitamin A in fish liver oils by ascorbic acid was suggested by Vedder and Rosenberg (16). The amount of excess used by these authors with rats was 200 times greater than was used in the present study of dogs, and the relationship which they suggested is not wholly consistent with the findings on these dogs. They found that excess vitamin D tended to decrease the toxicity produced by feeding jewfish liver oil in sufficient amount to provide 100,000 I.U. vitamin A per 50 gram rat per day.

TABLE 2

*Liver and kidney stores of vitamin A of dogs as affected by intake and by vitamin D medication*

DOG	VITAMIN D STATUS	WEIGHT OF KIDNEY	VITAMIN A PER GM. KIDNEY	TOTAL KIDNEY VITAMIN	WEIGHT OF LIVER	VITAMIN A PER GM. LIVER	TOTAL VITAMIN A LIVER	TOTAL VITAMIN A		TOTAL VITAMIN A
								Intake	Stored	Stored
		gms.	I.U.	I.U. $\times$ 1000	gms.	I.U.	I.U. $\times$ 1000	I.U. $\times$ 1000	I.U. $\times$ 1000	per cent of intake
1	Normal	57	550	31	286	400	114	1,770	145	8.2
10	Excess	33	275	9	143	150	21	965	30	3.1
4	Excess	46	225	10	194	225	43	1242	53	4.2
6	Normal	57	2,950	168	233	31,000	7,223	20,000	7,391	36.8
3	Excess	49	3,000	147	210	29,500	6,195	14,000	6,342	44.0
7	Excess	55	5,900	324	197	37,500	7,387	18,900	7,711	40.8
11	Excess	55	2,800	154	135	41,000	5,535	15,500	5,689	36.7

This is similar to the finding, discussed later in this report, of some protective effect of vitamin A excess against vitamin D overdosage effects.

*Hemoglobin.* When the animals were sacrificed, hemoglobin determinations were made by the Newcomer method (17). Dogs 1, 10 and 4, the low vitamin A group, had 16.9, 16.7 and 16.9 gram hemoglobin per 100 grams blood. Dogs 6, 3, 7 and 11, the high vitamin A group, had 15.8, 15.8, 14.0 and 15.6 grams per 100 grams blood. This difference, about 1 gram, in favor of the former may not be significant. It was not due to dehydration however since the hematocrits were comparable.

*The cause of the calcemia.* The degree of calcemia did not alone govern the calcification of tissues, the gross symptoms nor the growth of these dogs. Dog 7 which maintained the highest serum calcium in his group throughout the latter half of the experiment had less tissue calcification than nos. 3, 4, and 10 which had lower serum calcium. When no. 7 was induced to increase his food intake by addition of 30 grams of wheat germ daily to the diet, both his weight and serum calcium increased markedly (fig. 1 and 2). When his appetite decreased,

both rate of growth and serum calcium declined. This was taken to mean that the increased food intake was the cause of the rise in serum calcium and that perhaps partly as a result of this rise, eventually the toxic symptoms recurred. Similar but less striking changes were seen in no. 4, also given wheat germ, and in nos. 3 and 10 given similar amounts of yeast. This is in accord with the experience of many investigators, who have seen the effects of excessive vitamin D exaggerated by increases in calcium and phosphorus intake. Thus, the source of the extra serum and tissue calcium in these dogs may well have been the food rather than the skeleton, as has been suggested by the work of Morgan et al. (18) with vitamin D and parathyroid extract. Taylor and Weld (19) on the contrary believed the extra calcium to be withdrawn from the skeleton when excess vitamin D was given. But the amounts of dietary calcium used by the latter investigators were small, varying from 1 to 144 mgm. per dog per day. There was evidence in the present study of overmineralization of the long bones, rather than of decalcification and the calcium intake was 190 to 480 mgm. per kgm. per day.

*Toxicity of the various forms of vitamin D.* The moderate degree of toxicity produced by the excess tuna liver oil in dog 11 illustrates the difficulty involved in comparing pharmacological effects of vitamin D in terms of antirachitic units. At low levels, tested on rats, the tuna liver oil had effects which were comparable with those of the irradiated ergosterol, the other fish liver oils and the irradiated animal sterols (delsterol), but at the excess levels were much less pronounced than those of either the delsterol or the irradiated ergosterol.

The delsterol, presumably containing largely irradiated 7-dehydrocholesterol, had a far more rapid and severely toxic effect on dog 12 than did the equal dosage of vitamin D, partly at least  $D_3$ , administered in tuna liver oil to no. 11. The same difference was noted by Morgan, Shimotori and Hendricks, (17), in rats and was ascribed by them to the more rapid and complete absorption and utilization of the potent principle of delsterol. Dogs 7 and 13 which received irradiated ergosterol were less severely affected than no. 12 but more severely than dog 11. Earlier work with rats (4) had indicated similarly exaggerated effects of the same unitage, in terms of antirachitic activity, of irradiated ergosterol as compared with tuna liver oil. Harris, Ross and Bunker (20) reported similar observations. Jung (21), using crystalline vitamins  $D_3$  and  $D_2$  and carefully irradiated ergosterol, found no difference in their toxicity for rats but greater toxicity was obtained from similar doses of irradiated 7-dehydrocholesterol. Essentially the same conclusions as to the effects of vitamins  $D_2$  and  $D_3$  were reached by McChesney and Messer (22) as to hypercalcemia in dogs, and McChesney (23) as to toxicity for rats.

Our experience with these three forms of vitamin D would indicate that for young dogs excess dosage of the vitamin of tuna liver oil was least toxic, irradiated ergosterol intermediate and delsterol (irradiated animal sterols) was most toxic.

*The effects of excess vitamin A.* There were no adverse effects due to the continuous feeding of 10,000 I.U. vitamin A per kgm. per day in any dog. In

all of the three pairs of normal litter mates, nos. 1 and 2, 5 and 6, 8 and 9, the animal which received the excess vitamin A grew to a larger adult size (fig. 1). Of the seven dogs given excess vitamin D, the five which received equal amounts of vitamin D<sub>2</sub> may be compared. Three of these, nos. 3, 7 and 13 received the excess dose of vitamin A, 10,000 I.U. per kgm. per day, and two, nos. 4 and 10 received the lower dose of vitamin A, 800 I.U. per kgm. per day. The two males, nos. 7 and 13, were quite comparable as to growth, appetite, and calcification of tissues, and in all these respects were more nearly normal than the females 4 and 10. The female 3 proved an exception in that her condition was possibly the worst of all, even though she had received the larger amount of vitamin A. As noted previously, this animal was a premature and reacted to the treatment most unfavorably at all times.

The two remaining dogs, nos. 11 and 12, were assigned to receive the same treatment, that is, 10,000 units vitamin D<sub>3</sub> and 10,000 units vitamin A per kgm. per day. It is obvious, however, that their reactions were quite different, dog 11 on tuna liver oil showing little disturbance in growth, blood and tissue composition, and dog 12 on delsterol and shark liver oil, serious symptoms of anorexia, nausea, diarrhea and collapse. Even after 146 days' recovery in the case of the latter, extensive calcification of kidney, lungs and stomach remained. His littermate, no. 13, treated similarly but given vitamin D<sub>2</sub>, exhibited far smaller calcified residues in kidneys and lungs but a larger amount in the stomach.

These differences in the toxicity of the forms of vitamin D make it difficult to assess the effects of the excess vitamin A fed these dogs. The comparison must be confined therefore to no. 3 vs. no. 4 and no. 7 vs. no. 10. Dog 13 confirmed the findings in no. 7 but cannot be included in this group because of the recovery period allowed him. Again, since no. 3 was a premature, and apparently unusually susceptible to the vitamin D treatment, her response was unfavorable. Her condition was no better than that of no. 4. Dog 7 was in much better condition at all times than was no. 10. There can be only a tentative conclusion that the excess vitamin A may have had some protective effect against the excess vitamin D.

*Comparison with single massive dose treatment of rickets.* The total excess dosage given five of these dogs over 296 days was 2,360,000 I.U. vitamin D per kgm. or about four times the amount usually recommended (24) for the single dose treatment or for prophylaxis of infantile rickets. Two of the dogs were given 1,270,000 and 1,470,000 I.U. per kgm. over 360 days, yet the condition of their tissues was not much better than that of the former group. The dosage advocated for infants, 600,000 I.U. or 15 mgm., represents usually 100,000 to 200,000 I.U. per kgm. body weight but is given in one dose, as compared with the 6 to 24 times this amount given these young dogs over 296 days. The cumulative effect of the repeated moderately excessive dosage was on the whole not as severe as was the effect of one massive dose, 500,000 I.U. given another group of similar dogs (25) nor was recovery any more complete after 241 days in the 3 animals of the latter group than in the two allowed 146 days' recovery in the former group.

Some question as to the unusual susceptibility of premature infants to hy-

pervitaminosis D as well as to rickets may be raised. Zelson (26) treated 46 premature infants with single doses of 600,000 units of various vitamin D preparations orally and parenterally and reported that protection from rickets resulted. However only 17 infants were followed at all and only one for more than 62 days, most of them for only 30 days. Rickets or symptoms of late toxicity might have occurred in some of these cases. Certainly the animal judged to be premature in this series was very adversely affected by the treatment.

The apparently greater toxicity of the moderately excessive amount of vitamin D used in this experiment as compared with that described in earlier reports may probably be ascribed to the greater calcium content of the diet, the youth of the animals used and the longer period of medication.

#### SUMMARY

Thirteen purebred cocker spaniels reared in the laboratory colony were placed at weaning on a purified diet containing 1.0 per cent calcium and 0.73 per cent phosphorus. Seven were given 10,000 I.U. vitamin D per kgm. per day as irradiated ergosterol, delsterol or tuna liver oil and six were given 72 I.U. Some of each group had 800 and some had 10,000 I.U. vitamin A per kgm. per day.

The treatment was continued for 8 to 10 months when the animals were sacrificed and tissues examined. Two of these dogs after three or four months were allowed to recover for 146 days without further medication.

All dogs which received the excess dosage exhibited some symptoms of toxicity but the one which received the tuna liver oil had less, and the one which received delsterol had more, than the others which received irradiated ergosterol. Of the latter group, two of which were given the larger amount of vitamin A had less calcified soft tissues, better growth and less deformity of jaws and teeth than the two which received the smaller amount of vitamin A, but the fifth animal, apparently a premature, which received the larger dosage of vitamin A, showed the most abnormality of all.

The storage of vitamin A in kidneys and livers corresponded with the intakes, but was not affected by the excess vitamin D treatment. The ascorbic acid content of the adrenal glands but not the weight of those glands was greater in the dogs given the excess vitamin A than in those on the lower vitamin A intake, regardless of excess vitamin D intake.

The two dogs which were relieved of the medication exhibited good functional recovery as to appetite and growth but no repair of damage to teeth and jaws was noted. The animals showed no evidence of gross calcification of the hearts and muscles when sacrificed, but stomachs, lungs, and kidneys retained excessive amounts of calcium.

The growth of the dogs given excess vitamin D was inferior, nearly all the soft tissues were obviously calcified, the long bones were excessively mineralized and the shafts increased in thickness. The teeth were small, the roots deformed, pulp stones were common and the gum tissue inflamed.

The serum calcium level was raised in all the hypervitaminotic animals, but was variable and appeared to be governed by the calcium intake.

It was evident that under these conditions the vitamin D of tuna liver oil had

less toxic effect than that of irradiated ergosterol, but delsterol (irradiated animal sterols) had greater toxicity than either of the other two forms. Some evidence of alleviation of the hypervitaminosis D by intake of excess vitamin A was found.

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# THE EFFECT OF A SINGLE MASSIVE DOSE OF VITAMIN D<sub>2</sub> ON YOUNG DOGS<sup>1</sup>

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Increasing interest in the use of single large doses of vitamin D for the prevention of rickets in infants brings with it the need for more knowledge of possible undesirable sequelae of such treatment. Questions which need to be answered concern the immediate and later effects on serum, bones, teeth and soft tissues, the rate of removal of any excess calcification, the relative sensitivity of males and females and the differences in specific symptoms caused by the various forms of the vitamin. In particular, the relationship between serum calcium level and the other results of vitamin D shock treatment is of interest since such treatment is usually controlled in the clinic by serum calcium determinations.

Many studies, often reviewed (1, 2), have indicated the conditions which affect the manifestations of excess dosage of vitamin D. The short time clinical observations available appear to justify the use of large single or even repeated large doses of the vitamin particularly for the prevention or cure of rickets (3). A limited amount of attention has been given to the possible variation in toxicity of the different forms of vitamin D (4, 5) and to the protective effect of vitamin A (5, 6). Dependence upon rise in serum calcium as an index of hypervitaminosis D has developed even though there are indications that such changes may not always parallel the tissue changes. (1). The present study is reported because it indicates a new direction which the effects of such hypervitaminosis may take, namely, malformation of the teeth and jaws. The incidence of this malformation and its severity appeared to be related to the magnitude and duration of the hypercalcemia.

The remote rather than the immediate effects of the medication were studied in this experiment since few long time studies of the effects of single massive doses of the vitamin have been recorded. The diet used was of relatively high calcium content but normal Ca:P ratio. The amount of vitamin D<sub>2</sub> given was close to the minimum stated by Goormaghtigh and Handovsky (7) and by Dale, Marble and Marks (8) to be lethal for dogs, 12 mgm. (480,000 units) vitamin D<sub>2</sub> per kgm. body weight.

An earlier experiment (5, 9) in which chronic moderate overdosage of similarly handled young dogs had resulted in underdeveloped and maloccluded jaws, formation of pulp stones, calcification of periodontal membrane and gum tissue, suggested the desirability of a study of the effects of one massive dose of the vitamin. The total amount of vitamin D given the animals in the earlier experiment was equal to about 60 mgm. calciferol per kilogram body weight in a period of 10 months. The pathological changes were less severe in the dogs

<sup>1</sup> This study was supported by a grant from the Nutrition Foundation Inc., New York.

which received, in addition to the excess vitamin D, 10,000 I.U. vitamin A per kgm. than in those which received only 800 I.U. vitamin A per kgm.

**METHODS.** Eight purebred cocker spaniels from two litters born in the laboratory colony were weaned at the age of 3 to 4 weeks, and fed the stock diet made up as follows: casein 45.8, cornstarch 20.3, hydrogenated cottonseed oil 10.0, wheat germ 10.0, brewers' yeast 10.0, salts no. 5 (5) 2.4, calcium carbonate 1.5. This diet contained 1.23 per cent calcium and 0.78 per cent phosphorus. The dogs were also given 1 gram per day cod liver oil reinforced with carotene, providing 100 I.U. vitamin D and 2250 I.U. vitamin A. The calcium and phosphorus contents of this diet were higher than those usually used in such experiments (4, 10) but were chosen since the Ca:P ratio, 1.5:1, was nearly that of cow's milk, and the actual amount of these elements per gram of dry food was also close to that of milk.

TABLE 1

*Survival of young dogs given one dose, 450,000 I.U. vitamin D<sub>2</sub> as irradiated ergosterol*

DOG NUMBER	SEX	LITTER	AGE WHEN DOSE WAS GIVEN	WEIGHT WHEN DOSE WAS GIVEN	VITAMIN D <sub>2</sub> GIVEN	SURVIVAL PERIOD		
						After medication	Age at death	Age when sacrificed
			<i>days</i>	<i>kgm.</i>	<i>I.U. per kgm.</i>	<i>days</i>	<i>days</i>	<i>days</i>
14	Male	D	34	1.43	314,000	241		275
15	Female	D	34	1.30	346,000	37	71	
16	Female	E	29	0.81	556,000	2	31	
17	Female	E	29	0.85	530,000	2	31	
18	Male	E	29	1.05	429,000	14	43	
19	Male	E	29	1.10	410,000	88		117
20	Male	E	29	0.95	473,000	241		270
21	Male	E	29	1.10	410,000	241		270

The irradiated ergosterol<sup>2</sup> contained 800,000 I.U. vitamin D<sub>2</sub> per gram, but presumably contained also some tachysterol which although not antirachitic in therapeutic doses may contribute to calcemia and toxicity in massive doses. No toxisterols were present.

Although 100 I.U. vitamin D as cod liver oil had been given the dogs daily since they were 10 days of age, knobby ankles and flat feet developed in some of them and it was assumed that some degree of rickets was present. They were each given, when 29 or 34 days old, one dose of the irradiated ergosterol containing 450,000 I.U. vitamin D. Since the weights varied the dose per kgm. varied as shown in table 1. Grayfish oil, containing practically no vitamin D was given thereafter instead of the cod liver oil in an amount providing 2,000 I.U. vitamin A per kgm. per day.

The hearts, lungs and kidneys at autopsy were weighed, dried, ashed and Ca and P determined. Calcium was determined in the serum and ashes of organs and excreta by the method of Larson and Greenberg (11), inorganic phosphate

<sup>2</sup> The irradiated ergosterol in oil was supplied by Mead Johnson and Co. of Evansville, Indiana.

by that of Fiske and Subbarow (12). Samples of soft tissues, the entire tibia and the head were saved for roentgenological and histological study.

Immediately following the administration of the vitamin preparation there occurred in all cases bloody diarrhea, loss of appetite, and excessive thirst. In two days the two smallest females, nos. 16 and 17, had died. At this time 1,000,000 I.U. vitamin A<sup>3</sup> was given by mouth to each of the remaining six dogs to minimize if possible the effects of the excess vitamin D<sub>2</sub>. Including this dose the surviving dogs in all received 2700 I.U. vitamin A per kgm. per day.

Two weeks later a male, no. 18, also died. Probably no. 15 would have succumbed at this time also had she not been given special care in feeding for the following three weeks after which interval she also died. The four remaining dogs were maintained on stock diet with adequate amounts of vitamin as described, but one, no. 19, was killed at four months of age, 88 days after the dose of vitamin D<sub>2</sub> was given, to determine the extent of calcification of the soft tissues and to preserve the jaw with the deciduous teeth. This animal was selected for sacrifice because its condition was intermediate between those of 14 and 20, which were representative of the surviving group. The other three males were kept for 241 days until the permanent teeth were erupted and the jaws were completely developed.

*Effect on jaws and teeth.* Severe malocclusion bringing about a prognathous formation occurred in the jaws of all the young dogs. The deciduous teeth were abnormally placed in the jaw, and imperfectly erupted. The permanent teeth of the surviving dogs were smaller than normal, pitted, irregular in spacing in the jaws and covered by calculus. Of the 3 surviving dogs, no. 14 had most nearly normal teeth, no. 20 and no. 21 about equally maloccluded. Dogs 15 and 16 which were autopsied 37 and 88 days after medication had the most extensive evidence of malformation of jaws and teeth. These observations confirm those previously noted (9). A full report of the roentgenographic and histologic study of jaws and teeth of the dogs used in this experiment has been published separately (13). Osteoporosis of the mandibles, pulp stones and deformities of the roots were observed in all cases, but the most striking changes were seen in the dogs which survived for the shorter periods.

On autopsy, metastatic calcification of the heart, kidney, lung and brain, hemorrhage in the eyes and brain, and cataract in one eye were found in dog 15. In dog 19, killed 88 days after dosing, there was less calcification visible on gross examination of the organs, but chemical analysis revealed abnormally high calcium content of the lung, heart, and kidney. The liver appeared to be unaffected in any case.

Dog 14 was most nearly in normal condition. The malocclusion of the jaws was least pronounced, metastatic calcification of the soft tissues was not present, the Ca:P ratio of these tissues was nearly normal. Dogs 20 and 21 were intermediate between 14 and 19 as to calcification of organs and condition of jaws and teeth.

<sup>3</sup> Distilled vitamin A concentrate, supplied by Distillation Products, Inc., Rochester, New York.

*Serum calcium.* Serum calcium was determined<sup>4</sup> on only one of the 4 dogs which died, no. 15, a female which succumbed after 37 days and only once on a fifth dog, no. 19, which was sacrificed after 88 days.

Between the 105th and 190th days after the medication the calcemia of the surviving animals was constant, but by the 240th day the serum calcium level of no. 20 had returned to normal and that of the other 2 had dropped to nearly normal (fig. 1). The general condition as evidenced by anorexia, diarrhea, thirst, polyuria and muscular weakness was most severe in no. 21, intermediate in no. 20 and least severe in no. 14. Calcemia was also more marked in no. 21 than in either of the others. These symptoms persisted in all of the dogs throughout the survival period although they were least evident in no. 14. Dog 21 was extremely sensitive to lack of water and became nauseated and constipated if the water intake was restricted.

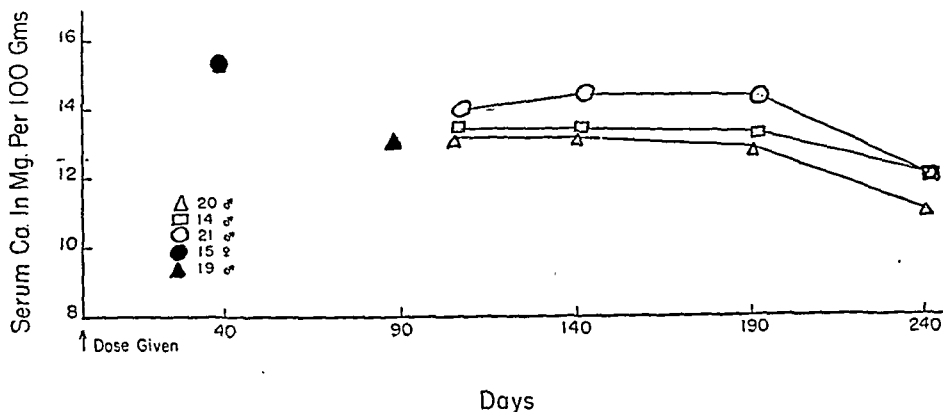


Fig. 1. Serum calcium of dogs as affected by one dose of vitamin D<sub>2</sub> as irradiated ergosterol.

The persistence of the calcemia for at least 190 days confirms the experience of Morgan and Shimotori (14) who noted in a similar young dog given one moderate dose of irradiated ergosterol 20,000 I.U. per kgm., serum calcium levels above 12 mgm. per cent for 90 days but for much shorter periods in dogs given the same amount of vitamin D in delsterol (irradiated animal sterols) or in tuna liver oil.

*Calcium and phosphorus retention.* Calcium and phosphorus balance studies were made 189 and 238 days after the medication on the 3 surviving dogs. The urinary Ca and P (fig. 2) appeared to increase when the retention was low as in the second balance on dog 20. This animal apparently was still retaining Ca and P in large amount during the first collection period, but 2 months later the retention was decreased. Dogs 14 and 21 maintained good retentions in both periods but during the second, dog 21 was failing in appetite and activity. This may mean that in these 2 animals the decalcification process, or at least a halt in the excessive retention, was under way before the first balance was made. It is interesting to note that the serum calcium of all 3 dogs was lower during the

<sup>4</sup> The serum and tissue analyses were performed by Olga Nave and Nobuko Shimotori.

second period (fig. 1) when the intake had dropped than during the first balance period. This would seem to confirm the findings in the earlier study (5) that rate of consumption of this diet, rich in Ca and P, affected the calcemia.

*Calcification of soft tissues.* The lungs of all the dogs were found to be more severely calcified than the kidneys and hearts (fig. 3). This is in accord with the

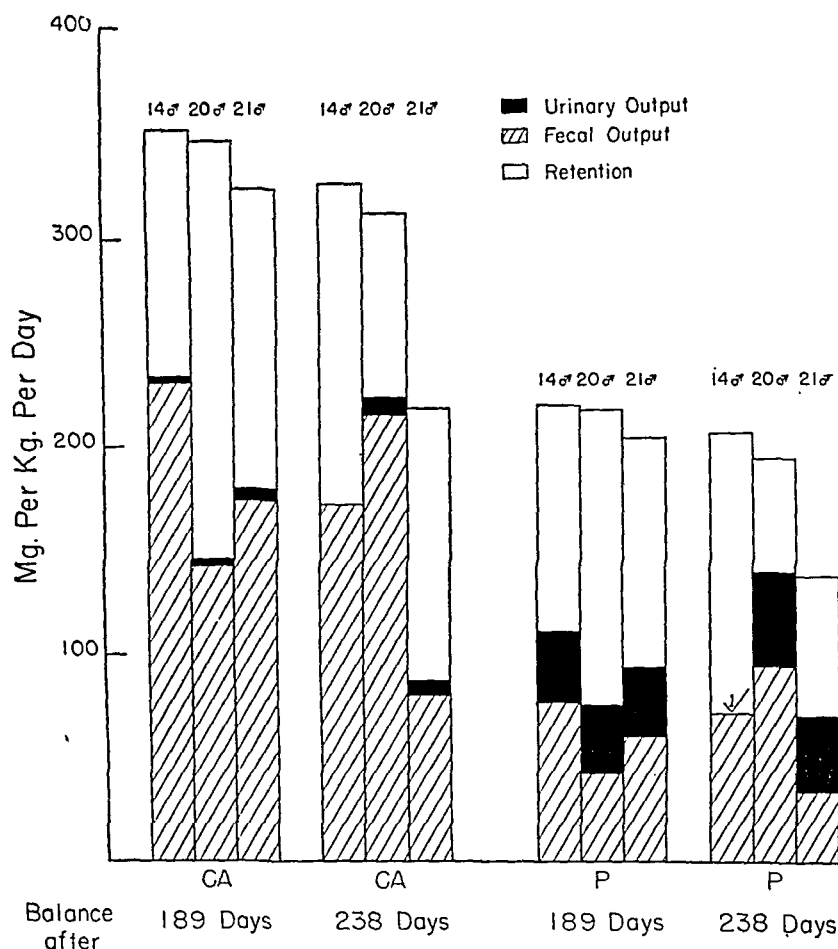


Fig. 2. Ca and P intake and excretion after administration of one large dose of vitamin D<sub>2</sub>. The numbers of the dogs are placed above the columns representing their balances. The urine of dog 14 was lost in the 2nd balance. This invalidated the P but not the Ca balance.

earlier finding (5) in two dogs allowed to recover for 146 days from chronic moderate overdosage with vitamin D.

The tissues of dogs 15 and 19 which lived 37 and 88 days after the medication contained more Ca and P than those of the 3 dogs which survived 241 days. The tissues of dog 21, which maintained the highest serum Ca level of these three, were on the whole not much more calcified than those of dogs 14 and 20 (13).

*Influence of sex.* Of the 4 dogs which died of the overdosage, 3 were females.

Two of these were somewhat smaller (table 1) than the males but one was larger than all but one of the males at the time of the medication. According to McLean (2) "the effectiveness of the calcemic principle, common to vitamin D and dihydrotachysterol, is affected by the degree of parathyroid insufficiency, by the intake of calcium and phosphorus, by the female hormone, and perhaps by other physiologic variables. . . . The calcemic principle is identical with the factor responsible for the toxic manifestations of overdosage." Whether the estrogenic hormones were present in sufficient amount in these young dogs to increase the severity of the condition is open to question. Sos, Lichner

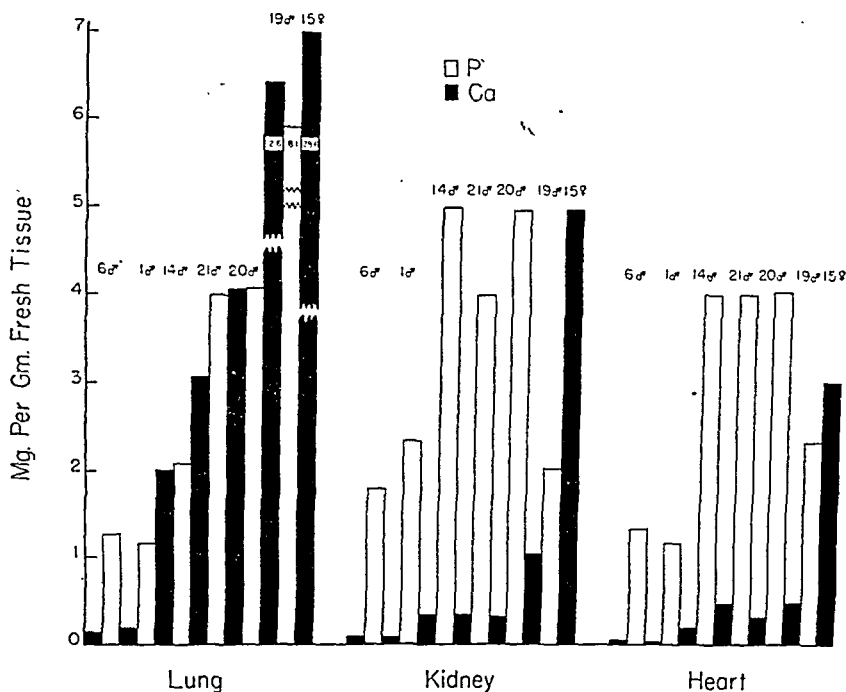


Fig. 3. The Ca and P content of lungs, kidneys and hearts of young dogs given one large dose of vitamin D<sub>2</sub>. The numbers of the dogs are placed in or over the columns representing their tissue calcium. Dogs 1 and 6 were normal animals of the same age and strain as the others and were fed the same diet (5).

and Ats (15) reported that oestrone reduced the mineral content of bones of rachitic rats when no vitamin D was given but that dihydro-oestrone benzoate and stilbestrol had no such effect. Jung (16) found female rats more sensitive than males to cumulative vitamin D overdosage but Morgan, Kimmel and Hawkins (17) concluded that in rats there was no difference between the sexes in this respect.

It is evident that the single dose given these young dogs was larger than that usually recommended for the vitamin D-Stosse prevention of rickets in infants. The latter is 600,000 I.U. vitamin D given to infants weighing 3 to 6 kgm. (3), or 100,000 to 200,000 units per kgm. The dogs were given 300,000 to 500,000

units per kgm. The margin of difference is not so large however as to warrant complete confidence in the safety of the vitamin D-Stosse procedure.

#### SUMMARY

Of 8 young dogs, 4 to 5 weeks old, given a single dose of vitamin D as irradiated ergosterol, 314,000 to 530,000 I.U. per kgm. 3 were dead within 2 weeks and a fourth was moribund in 5 weeks. All exhibited the usual symptoms of overdosage, anorexia, polyuria, bloody diarrhea, excessive thirst and prostration.

The serum calcium remained elevated for 6 months in the 3 animals which were observed during a period of 241 days. Calcium and phosphorus excretion after 189 and 238 days indicated continued retention of these elements.

Extensive calcification was found in the lungs and moderate calcification in hearts and kidneys of all animals, but the excess calcium deposition was more striking in the 2 animals which succumbed or were sacrificed soon after the medication. Evidently decalcification of the soft tissues took place gradually during the recovery.

Malocclusion, pitting, irregular placing and poor development of the teeth were seen in all the dogs and these conditions were not appreciably improved during the recuperative period.

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# THE DISTRIBUTION OF SODIUM AND POTASSIUM IN OXYGENATED HUMAN BLOOD AND THEIR EFFECTS UPON THE MOVEMENTS OF WATER BETWEEN CELLS AND PLASMA

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The membranes of "resting" human red blood cells appear to be impermeable to cations other than hydrogen and ammonium. Wakeman, Eisenman and Peters (1) found that, if the bicarbonate or chloride of either potassium or sodium was added to blood in the resting state, chloride and bicarbonate distributed themselves between the two media in a characteristic manner, but the quantities of total base and sodium in the cells did not change. In a subsequent study Danowski (2) showed that potassium was transferred between cells and plasma during metabolic activities of the cells, the direction of its movement depending upon the nature of these metabolic activities. Eisenman, Ott, Smith and Winkler (3) with the aid of radioactive isotopes of sodium and potassium demonstrated that in resting blood equilibrium was not established between potassium in the cells and potassium added to blood. Results with sodium were somewhat equivocal. This element did not seem to be as completely excluded from the cells as potassium was; but it was impossible, for technical reasons, to determine whether equilibrium was established between the sodium of cells and plasma.

The present study was undertaken to explore with better analytical procedures and under more rigorous conditions the forces that restrain the passage of base across the membranes of the red blood cells.

**EXPERIMENTAL.** The experiments follow the general procedure used by Wakeman, Eisenman and Peters (1). Blood was defibrinated in an open vessel by means of a glass rod. It was then equilibrated with an atmosphere of 40 mm. of CO<sub>2</sub> in air at 37°C. before and after the addition of known amounts of sodium chloride, sodium bicarbonate, sodium sulfate, potassium chloride, potassium bicarbonate water or 9 per cent sucrose solution. The techniques employed in the preparation of the blood have been described in a previous paper (4). Cell volume was measured in all experiments. Water of serum and blood was either measured gravimetrically or calculated from the nitrogen of blood and serum or from hemoglobin and serum protein. Hemoglobin was estimated either from oxygen capacity or from cell nitrogen. Methods of calculation have been described (4).

<sup>1</sup> The term "resting" is applied to the state in which the metabolic processes within the cells are reduced to a minimum, either by keeping the blood at refrigerator temperature or by completing a procedure so rapidly that the metabolic activity during the interval is negligible.

In 8 experiments which are presented in detail in tables 1 and 2, both blood and serum were analyzed for sodium and potassium by the method of Hald (5).

TABLE 1

*Analyses of blood and serum before and after the addition of salts\**

NO.	TREATMENT OF BLOOD	CELL VOLUME PER CENT	WATER		SODIUM		POTASSIUM	
			Serum per cent	Cells per cent	Blood mM. per l.	Serum mM. per l.	Blood mM. per l.	Serum mM. per l.
1.	Original	38.5	93.2	71.6	90.2	133.5	42.0	3.9
	After NaCl	30.5	93.9	64.0	160.2	215.9	42.0	3.3
2.	Original	45.0	93.5	72.6	81.5	136.0	47.2	4.3
	After KCl	35.6	94.4	65.3	81.5	121.5	117.2	107.6
3.	Original	42.3	93.3	73.2	83.3	134.5	42.7	4.2
	After Na <sub>2</sub> CO <sub>3</sub>	35.6	94.0	68.2	141.1	195.5	42.7	3.7
4.	Original	28.2	93.1	75.1	101.5	146.3	35.0	4.9
	After NaCl	22.2	93.6	68.4	175.7	221.0	35.0	5.1
5.	Original	40.6	93.2	70.4	88.0	139.1	40.1	4.3
	After NaCl	31.7	94.2	62.0	158.1	222.5	40.1	3.5
6.	Original	39.9	93.2	72.1	79.5	124.5	41.2	3.9
	After Na <sub>2</sub> CO <sub>3</sub>	31.0	94.2	64.2	148.4	199.1	41.2	5.1
7.	Original	45.0	92.9	70.4	87.5	136.3	40.3	4.0
	After NaCl	34.9	93.5	61.3	158.6	235.0	40.3	3.7

\* All specimens, both before and after additions of salt, were equilibrated with 40 mm. of CO<sub>2</sub> in air at 37°.

TABLE 2

*Distribution of sodium and potassium between the water of blood cells and serum, calculated from data of table 1*

NO	TREATMENT OF BLOOD	[Na]		[K]		[Na + K]		D <sub>Na+K</sub>	AMT. IN CELLS		CHANGE	
		Serum mM. per l.	Cells. mM. per l.	Serum mM. per l.	Cells mM. per l.	Serum mM. per l.	Cells mM. per l.		Na mM.	K mM.	Na mM.	K mM.
1.	Original	143.3	29.4	4.2	143.7	147.5	173.1	1.17	8.1	39.6		
	After NaCl	229.9	52.3	3.5	203.2	233.4	255.5	1.09	10.2	39.7	+2.1	+0.1
2.	Original	145.5	20.5	4.6	137.4	150.1	157.9	1.05	6.2	44.8		
	After KCl	128.8	27.6	114.0	206.1	242.8	233.7	0.96	3.3	47.9	-2.9	+3.0
3.	Original	144.2	18.4	4.5	130.0	148.7	148.4	1.00	5.7	40.3		
	After Na <sub>2</sub> CO <sub>3</sub>	208.0	63.0	3.9	166.1	211.9	229.1	1.08	15.3	40.4	+9.6	+0.1
4.	Original	157.2	-12.4	5.3	148.5	162.5	148.5	0.91	-3.5	31.5		
	After NaCl	236.0	24.4	5.5	204.2	241.5	228.6	0.95	3.7	31.0	+7.2	-0.5
5.	Original	149.4	19.3	4.6	131.6	154.0	150.9	0.97	5.5	37.6		
	After NaCl	236.1	31.0	3.8	191.9	239.9	222.9	0.93	6.1	37.7	+0.6	+0.1
6.	Original	133.5	16.4	4.2	135.0	137.7	151.4	1.10	4.7	38.9		
	After Na <sub>2</sub> CO <sub>3</sub>	206.5	55.8	5.4	189.2	211.9	245.0	1.16	11.1	37.7	+6.4	-1.2
7.	Original	146.8	40.1	4.3	119.3	151.1	159.4	1.05	12.7	37.9		
	After NaCl	251.6	25.5	4.0	172.7	255.6	198.1	0.78	5.6	37.9	-7.1	0

In 21 additional experiments serum alone was analyzed for total base or sodium or both by the methods of Hald (6).

Sodium and potassium of cells and whole blood of a large number of normal and diabetic subjects were also determined. The blood in every instance was treated with anaerobic precautions. With the exception of initial studies of patients with diabetic acidosis blood was drawn when subjects were in the postabsorptive state. The results of these analyses may be found in a paper by Hald (5) and in a subsequent paper of this series (7).

Including the experiments in table 1 there were altogether: 9 in which sodium chloride was added, 7 with sodium bicarbonate, 4 with potassium chloride, 2 with potassium bicarbonate, 1 with sodium sulfate, 3 with water (some water was also added in one of the sodium chloride experiments) and 3 with sucrose. The supplements of salt varied from about 10 to 75 mEq. of base per liter of whole blood. The experiments with the largest increments are shown in tables 1 and 2. The additions of water amounted to about 20 per cent of the volume of the whole blood; the additions of 9 per cent sucrose to about 50 per cent of whole blood.

**RESULTS.** In table 2 may be found data derived by calculation from the analyses in table 1. These experiments were chosen for presentation in detail because in them the largest amounts of salts were added to blood, because both potassium and sodium were measured and because both whole blood and cells were analyzed. In the first 4 columns are the concentrations of sodium and potassium in the water of cells and serum; in the next 3 are the combined concentrations of sodium and potassium in the two media, followed by the distribution coefficient—i.e., the ratio,  $[Na + K]_c : [Na + K]_s$ . In columns 8 and 9 are the amounts of Na and K in the cells of a liter of blood, obtained by multiplying the concentrations of these substances in the cells by the cell volume. The last 2 columns give the calculated changes in the amounts of sodium and potassium in the cells as a result of the experimental procedure.

In all of the 22 experiments in which salt alone was added to blood the cells contracted, demonstrating the general permeability of the membranes to water and their resistance to the passage of some components of the salts. The quantitative relation of the transfer of water to the osmotic effect of various added substances was tested in the following manner. It was assumed that the osmotic pressure of cells and serum is identical. The osmolar concentration in cells can not be evaluated from knowledge of their chemical composition. In serum, however, this approximates closely the sum of the concentrations of Na and K per unit of water, assuming that these ions are monovalent, completely ionized and osmotically active. Therefore,

$$[Na + K]_s^0 (W_s^0 V_s^0 + W_c^0 V_c^0) \text{ should equal } (Na + K)_b^0$$

in which  $[Na + K]$  indicates concentrations per unit of water,  $W$  = water,  $V$  = volume, the subscripts  $b$ ,  $s$ , and  $c$  represent blood, serum and cells, respectively, the superscripts  $^0$  and  $^1$  indicate untreated and treated bloods, and  $(Na + K)$  represents the total quantity of osmotically active material in the water of whole blood or serum. In the same way

$$[Na + K]_s^0 (W_s^0 V_s^0 \text{ should equal } (Na + K)_s^0$$

Furthermore,  $(\text{Na} + \text{K})_b^0 + \text{I} = (\text{Na} + \text{K})_b^1$

$$\text{and } (\text{Na} + \text{K})_s^0 + \text{I} = (\text{Na} + \text{K})_s^1$$

when  $I =$  the increment of base added to the blood. If the increment is completely dissociated into active ions, if neither sodium nor potassium cross the cell membrane (or if equal amounts of the two are reciprocally transferred), while water traverses it freely,

$$\frac{(Na + K)_s}{(Na + K)_b} \text{ should equal } \frac{W_s V_s}{W_c V_c + W_e V_e}$$

“calculated”                      “found”,

The 8 experiments of table 1 have been subjected to this treatment; in the other 15 experiments in which salt was added, total base was substituted for  $\text{Na} + \text{K}$  in the equations. The ratio, "found": "calculated" water of serum in the 19 experiments in which it could be calculated varied from 0.939 to 1.037, with a mean of 0.985. The 3 experiments in which water was added, when subjected to similar treatment, yielded ratios of 101.4, 103.6 and 99.8. Although some of the discrepancies in these experiments may be significant, their small size attests to the essential integrity of the assumptions. Potassium and sodium salts are probably not the only osmotically active components in serum, their salts are not entirely univalent and the inorganic cations and anions may not be always 100 per cent active. Furthermore, shifts of chloride and bicarbonate between cells and serum after the addition of salts may alter the concentrations of osmotically active anions in the cells. All these features, added to analytical errors, may be responsible for small divergences of the ratio from unity. There is no evidence in these experiments that the effects of potassium and sodium salts differ. Furthermore, the effects of sodium chloride and sodium bicarbonate on the redistribution of water are not clearly distinguishable in spite of the different effects of these salts on pH.

The experiments in the tables merit more particular examination because in these additions of salt were large and both Na and K were measured. In these the ratios, "found": "calculated" water in the two bicarbonate experiments were 0.939 and 0.966, while in the 5 chloride experiments they ranged from 0.964 to 1.035. A rise of pH from 7.4 to 7.9, which was produced in the bicarbonate experiments, if induced by lowering CO<sub>2</sub> tension, should cause a demonstrable contraction of the cells. In the present experiments the larger osmotic effect of the increment of base tends to obscure the effect of the change of pH. Calculations indicate, moreover, that there may have been an appreciable transfer of base from serum to cells after addition of bicarbonate, which would neutralize to some extent the effect of the change of pH. Whether this transfer of base is real or not, its effect on the estimation of "calculated" water would be the same. Finally it will be pointed out in a subsequent paper that bicarbonate causes an appreciable change in the state of cellular phosphate, while chloride does not.

The quantities of base in the two phases of the blood changed little, if at all, in any experiment. Calculations of the amounts of sodium in the cells before

and after additions of salts, water or sucrose solution in 20 experiments reveal changes of cellular sodium content varying from  $-7.1$  to  $+9.6$  mM., with an average of  $+1.7$  mM. There is reason to doubt the validity of 3 of the largest deviations. Two of these will be discussed below. The third belongs to the experiment in which the addition of both sodium chloride and water greatly complicated calculations. If these three are omitted the average transfer of sodium becomes  $+1.3$  mM. From total base in 19 experiments the average transfer is  $+1.6$  mM. with extremes of  $-3.1$  to  $+9.1$ . No consistent distinctions can be discovered in the whole group of experiments between the effects of different salts or water or sucrose solution. The tendency to a slight positive deviation is evident in all series. It probably arises, therefore, from some systematic error in calculation or analysis, since additions of water and sucrose reduce the concentrations of sodium and potassium in serum, while additions of salts increase them.

In the 7 experiments of tables 1 and 2, despite the drastic treatment to which the blood was exposed, no appreciable quantities of potassium crossed the cell membrane. About the experiment in which potassium chloride was added there may be some question, which will be discussed below. It is impossible to assert with equal assurance that sodium was excluded from the cells after addition of sodium salts. In experiments 3, 4 and 6 quantities of sodium that appear significantly large seem to have entered the cells, while in experiment 7 an equally large amount seems to have moved in the opposite direction. This inconsistency casts some doubt upon the validity of these transfers. It is inherent in the nature of the procedures that the greatest burden of error falls upon the estimation of sodium shifts because this requires the mathematical treatment of the difference between two large values by processes that involve other variables (cell volume, cell water and serum water) that are themselves subject to error. Experiment 4 contains an obvious error, since the figure for cellular sodium in the original blood is negative. If the value for sodium in the original serum is changed to a more plausible figure, based on the analysis of the final serum and the known amount of sodium chloride added to the blood, the quantity of sodium in the original cells becomes  $9.3$  mM. and the amount transferred is reduced from  $7.0$  to  $3.0$  mM., a quantity not outside of the error of the method. This leaves the bicarbonate experiments as the only two in which sodium appears to have entered the cells. The movement of sodium in the opposite direction in experiment 7 is at variance with all the other chloride experiments. It is suspect also because the sodium moves paradoxically against a large concentration gradient. Even if the data are accepted at their face value these small transfers can not be used as an argument for the simple diffusion of sodium across the membrane. The transfer of potassium in experiment 2 may be significant and connected with the fact that this is the only experiment of this series in which the concentration of potassium in blood was increased. It follows, however, that in this experiment alone, the error in estimating cellular potassium assumed the proportions encountered in the estimation of cellular sodium in other experiments. In the study by Wakeman, Eisenman and Peters (1) and in the other series of

present experiments total base concentrations in serum were affected identically by equivalent additions of sodium and potassium salts. This could be true only if no base crossed the membranes or if equivalent amounts of sodium and potassium were exchanged between cells and serum. No evidence of such a reciprocal exchange is seen in the experiments in tables 1 and 2, although the amounts of salt added were greater than those added in the other series. Unless or until analytical techniques can be further refined, therefore, it can not be denied that under the strain of great concentration gradients the impermeability of the resting red blood cell to sodium and potassium can be broken down, but it does seem clear that there is no simple diffusion equilibrium between extracellular and intracellular sodium or potassium. Furthermore, in resting blood potassium is excluded at least as efficiently as sodium from the cells, despite the fact that these cells exhibit a predilection for potassium.

The experiment with sodium sulfate and the 3 sucrose experiments introduce new variables. Sulfate is quite as effectively excluded from the cells as is the sodium with which it is combined (8). Furthermore, since sulfate is a strong bivalent acid, the osmotic effect of sodium sulfate, per equivalent of base is only three-quarters as great as that of chloride or bicarbonate containing the same amount of base. The movement of water which it provoked proved to be proportionally smaller than that induced by monovalent salts. When either salts or water are added to blood, by transfers of water between cells and serum, the proportions of  $\text{Na} + \text{K}$  in the two media are readjusted in such a way that the distribution of base is partially equalized. When isotonic sucrose is introduced no such shift of water occurs. The distribution coefficient of  $\text{Na} + \text{K}$  becomes grossly distorted without any change in the proportions of various electrolytic components in either cells or serum. All become equally diluted in the serum. At the same time the serum electrolytes become no longer a measure of osmolar concentration. Large and altogether abnormal concentration gradients are also set up without provoking any corrective reactions on the part of the electrolytes.

In the normal individual at rest in the postabsorptive state the ratio,  $[\text{Na} + \text{K}]_c : [\text{Na} + \text{K}]_s$  is greater than 1.0 and peculiarly constant. In a series of 8 normal subjects in the postabsorptive state this ratio remained within the narrow limits, 1.055 to 1.068 (5). The entire electrolyte patterns of such subjects vary within narrow limits.  $[\text{Na} + \text{K}]_s$  in the same series varied only from 148.2 to 154.9 mM. per liter. Nevertheless, no direct correlation can be found between the distribution coefficient and the concentration of monovalent base in serum. Although  $[\text{Na} + \text{K}]$  is normally greater in cells than in serum, the proportions of these bases in the two media in random bloods can vary greatly, their ratios even becoming inverted at times, without corresponding shifts of water (7). Neither in the normal subjects nor in patients to be presented subsequently (7) can any correlation be established between  $D_{\text{Na}+\text{K}}$  and the concentration of hemoglobin in the cells. In resting cells  $D_{\text{Na}+\text{K}}$  does appear to respond consistently to specific influences. In the present study, for example, this ratio fell in 5 of 6 experiments after additions of chloride, but rose in the two bicarbonate exper-

iments. It has already been pointed out that the one chloride experiment in which it rose is subject to correction.

DISCUSSION. These experiments confirm the general opinion that the membranes of the red blood cells permit the free passage of water, that the chief osmotically active components of the serum are monovalent salts of sodium and potassium (mainly chlorides and bicarbonates), that these salts are completely ionized, and that these ions are altogether or almost altogether osmotically active. Other things being equal, the volume and the water content of the cells will, therefore, vary inversely as the concentration of  $\text{Na} + \text{K}$  in the serum. It follows that the osmotic pressure—and, hence, the osmolar concentration of solutes—in the cells must equal that in the plasma. Nevertheless, the osmolar concentration within the cells is not so precisely proportional to the concentration of  $\text{Na} + \text{K}$  as it is in serum. Ordinarily this concentration is greater per unit of water in cells than in serum by a constant proportion.

In the eight normal samples of blood reported by Hald (5)  $[\text{Na} + \text{K}]_c$  is directly correlated with  $[\text{Na} + \text{K}]_s$ . In other words  $D_{\text{Na}+\text{K}}$  is more constant than is the concentration of  $\text{Na} + \text{K}$  in the water of either serum or cells. Furthermore,  $[\text{Na}]_c$  and  $[\text{K}]_c$  are inversely related; the concentration of  $\text{Na} + \text{K}$  in cells is more constant than that of either of its components. It may be inferred that among normal persons in the postabsorptive state the osmotic activities of sodium and potassium and the anions by which they are balanced are relatively constant and that the activities of sodium and potassium do not differ greatly. There is no evident reason, under these circumstances, why the cells should elect to carry a larger load of sodium in one person (or at one time, because personal idiosyncracies have not been investigated) than in another. Nevertheless, they do.

In the classical study of electrolyte equilibria in blood by Van Slyke, Wu and McLean (9) the distinctive patterns of bases in the two media of blood were neglected; the basic ions were treated as if all belonged to a single species. It was assumed that the base did not cross the cell membranes, which were also permeable to protein. It was also assumed that the osmotic pressure was the same in both phases of blood. This, as Wu (10) emphasized in a subsequent paper, was possible if the restraint upon the protein anion was balanced by a similar restraint upon the basic cations. The inequality in the distribution of base between the two media is not, therefore, like that between sera and transudates, directly dependent upon the Gibbs-Donnan equilibrium. The total osmolar concentration of a solution is the sum of the concentrations of three components: 1, active cations; 2, active anions; 3, nonelectrolytes. Osmotic equality is compatible with unequal concentrations of base, therefore, if a fraction of base is inactive, if the concentrations of cations and anions are not the same, or if there is a greater concentration of nonelectrolytes in one medium than in the other. Of the activity of sodium and potassium within the red blood cells nothing is known. Some part of either or both may be inactive, but it is not necessary to postulate inactivity of these elements to explain their disparate distribution in blood. Analyses indicate that there are larger quantities of

certain nonelectrolytes in cells than in serum. In serum there is no evidence of important quantities of inactive sodium or potassium and these cations are balanced chiefly by monovalent anions. In the cells, on the other hand, the anions are composed for the most part of proteins and organic phosphates. The former are undoubtedly, some of the latter are probably, multivalent. Under these circumstances, even if cations and anions in the cells were all osmotically active, osmotic equality between cells and serum could be maintained only if the cells contained either a greater concentration of base or a greater concentration of nonelectrolytes or both.

These principles are well illustrated in the experiments with sodium sulfate and with sucrose. The former, having a bivalent anion, placed less obligation upon water than did a chloride or a bicarbonate containing an equivalent amount of base. It therefore lowered the distribution coefficient of  $\text{Na} + \text{K}$  and reduced or reversed the usual disparity between cells and serum. The addition of isotonic sucrose solution increased this disparity enormously without disturbing osmotic relations or the distribution of water between cells and serum.

Under limited controlled conditions like those of the present experiments, in which osmotic relations are specifically strained, water moves in predictable proportion to  $[\text{Na} + \text{K}]_s$ .  $[\text{Na} + \text{K}]_c$  changes accordingly, but  $D_{\text{Na}+\text{K}}$  may vary greatly, even becoming inverted at times, without corresponding disturbances of the distribution of water between cells and serum. This will be illustrated in a subsequent paper (7). Again this lack of correlation could be attributed to changes in the activity of cellular base. In large part, however, it may be referable to changes in the nonelectrolytes which do not traverse the cell membranes and in the nature or combining powers of the anions. To such changes of nature and combining powers proteins and phosphates are peculiarly susceptible.

Conway (10) has proposed that the distribution and movements of water and electrolytes between muscle cells and their environment can be explained on principles of simple membrane equilibrium if it be assumed only that the membrane of the muscle cell is impervious to protein, sodium and organic phosphates. His experiments with isolated frog muscle are well contrived, his arguments are ingenious; but his assumptions are open to question and he has oversimplified his problem. His theory surely can not be applied to the red blood cell. In the first place this cell contains variable and far from negligible quantities of sodium. In some species, such as the dog, it contains almost as much sodium as potassium. Nevertheless, in the resting state these cells contract when either sodium or potassium salts are added to them. Despite its predilection for potassium the human cell, at rest, is no more receptive to this ion than to the sodium ion. It blocks its entry or exit against extreme concentration gradients. It will repel sodium against equally large gradients. It behaves in a similar manner towards inorganic phosphate, to which the membrane of the resting blood cell is equally impervious (7, 11). On the other hand, all these substances traverse the membrane of the active cell. If the ability of the cell is destroyed the selective characteristics of its membrane die with it.

Conway has dismissed the red blood cell from consideration as an effete member of the biological system. Nonetheless, it possesses the general characteristics of other cells. In some respects it has more universal properties than the highly specialized muscle cell that Conway pictures. It is not unique in containing a modicum of sodium and considerable chloride. It is unique among mammalian cells in certain other respects: it can be isolated for direct analysis, it is viable outside the body, and its metabolism can be reduced to minimal proportions and studied under controlled conditions.

So long as the blood cells are maintained in a resting state exchanges of water and electrolyte between them and the serum follow in a reasonably satisfactory manner the behavior of a system with the following characteristics: a membrane permeable to water,  $\text{CO}_2$ ,  $\text{Cl}$ ,  $\text{HCO}_3^-$  and hydrogen ions, but impenetrable to most other anions, particularly protein, and to bases, separating two compartments, one of which contains far more protein than the other. If, on the other hand, metabolic activities are proceeding in the cells, materials which ordinarily are restrained by the cellular membrane move across it freely. This would have to be if cells are to have origin and life. It has already proved possible with respect to the red blood cell to gain some insight into these movements. Individual components of the blood can be made to move in a predictable direction across the cell membrane by varying the metabolic activities of the cells.

Krogh (12) has recently attacked Conway's theory on similar grounds. Like the authors he recognizes that transfers of potassium and other materials between cells and their environment are linked with chemical reactions of metabolism that produce energy. If energy is introduced into a system such as cells and their environment the production of concentration gradients across membranes presents no insurmountable difficulties. There seems no good reason to exclude energy from consideration in systems in which it abounds. Nothing but a conventional attitude towards membranes prohibits the inference that, if transfers of certain solutes are associated with metabolic reactions, they may be implemented by the energy derived from these reactions. It seems to chemists an alien thought that a distinction should be drawn between simple inorganic elements, such as sodium or potassium, by a membrane or a protein. Nevertheless, such distinctions are regularly drawn by enzyme systems which have now been removed from the field of metaphysics. A study of the association of exchanges of electrolytes and other materials between biological media and chemical reactions within those media should be a profitable pursuit.

#### CONCLUSIONS

By the addition to blood of sodium and potassium chloride and bicarbonate, sodium sulfate, water, and 9 per cent sucrose solution, it has been shown that when metabolic activities of the red blood cells are in abeyance their membranes are almost, if not absolutely, impervious to both sodium and potassium even when concentration gradients of considerable magnitude are produced. The bearing of this fact on osmotic phenomena and on the distribution and exchanges of water and electrolytes has been discussed.

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# A SENSITIVE METHOD FOR THE ASSAY OF INSULIN IN BLOOD<sup>1</sup>

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The purpose of this communication is to describe a sensitive method for the detection of insulin in blood. Hemmingsen, Nielsen and Nielsen (1938) have shown that adrenalectomized mice develop hypoglycemic convulsions on doses of 0.0001 to 0.0002 unit of insulin per 10 grams of body weight. Gellhorn, Feldman and Allen (1941) have found the convulsant dose in hypophysectomized adrenodemedullated rats to be 0.001 unit per 100 grams body weight, and have suggested that the blood sugar response in this test animal be used as a method for measuring insulin in blood. Using adrenodemedullated diabetic hypophysectomized (ADH) rats, weighing approximately 200 grams, we have found that 0.00012 unit of insulin administered intravenously causes a significant lowering of the blood sugar. This method has been used by Anderson and Long (1947a, b, c) to detect insulin in the blood in connection with studies on the hormonal factors which influence insulin secretion. Since the ADH rat is so sensitive to exceedingly small amounts of insulin it has been found convenient to use the term milli-unit for 0.001 unit insulin.

**METHODS.** *Preparation of test animals.* The ADH rat, which was used as the test animal, was prepared as follows: The demedullation was done just after the rats had been weaned; at this age the operation takes less time because exposure of the adrenal is easier, and fewer stitches are required to close the wound. The operation consisted of nicking the adrenal capsule and squeezing its contents out through the opening. It has been shown by Evans (1936) that in 10 to 18 days after such a procedure the glands have regenerated, with their structure consisting of newly formed cortical cells in columnar arrangement; no chromaffin tissue was present.

When such rats had reached a weight of 225 to 250 grams they were made diabetic by the administration of alloxan. The alloxan was given intraperitoneally in doses of 12 mgm. per 100 grams body weight per day for 2 or 3 successive days. On the third day, before the last dose of alloxan was to be given, a blood sugar determination was made. If the blood sugar was 350 mgm. per cent or more the third dose of alloxan was omitted. At least 10 per cent of the animals were rendered diabetic after the second injection of alloxan. Of the group which were given the third dose of alloxan, about 10 per cent became se-

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<sup>2</sup> Fellow of the John Simon Guggenheim Memorial Foundation for 1946-47.

verely diabetic. The average blood sugar was approximately 500 mgm. per cent; however, an elevation of the blood sugar up to 900 mgm. per cent often was found.

The mortality from alloxan administration was greatly reduced by the forcing of fluids during the 3 days of alloxan injection, each rat being given 15 cc. of 1.0 per cent NaCl solution intraperitoneally twice a day, and in place of the regular food and water, a 10 per cent solution of sucrose to drink. Altogether each rat had at least 50 cc. of water per 24 hours.

The animals were started on protamine zinc insulin (PZI) as soon as the diagnosis of a severe diabetes was made, usually 24 hours after the last dose of alloxan. The dose of PZI was 2 to 3 units a day; failure to gain weight was an indication for increasing the dose.

Hypophysectomy was performed when the rats had regained their weight to the prediabetic level. This required at least 10 days of insulin therapy. A striking increase in insulin sensitivity of the animal occurs within an hour after hypophysectomy. Because of this no PZI was given in the 24-hour period preceding operation. Following hypophysectomy the animals appeared to be in a satisfactory state of health without insulin therapy. One or two weeks after the operation they were ready for use as test animals. Each animal was used once or twice a week. On most of the animals 4 assay tests could be run, and occasionally an animal could be used as often as 10 times.

*Procedure of assay test.* The method consisted of the giving of a glucose meal followed by an intravenous injection of the material to be assayed. A measurement of the insulin effect was based on a change in the blood sugar level over a 30-minute period. An outline of the procedure is as follows:

1:00 p.m.	5 cc. of 20 per cent glucose solution, warmed to body temperature, was given by stomach tube. Nembutal, 0.32 mgm. (0.05 cc. of 6.5 per cent solution), was then given subcutaneously, after which the animal was placed in a warm chamber at 100°F.
1:25	The animal, under light anesthesia, was placed on an operating board; the area over the jugular vein was shaved, an incision made, and the jugular dissected out.
1:29	1.5 cc. of plasma or 3 cc. of heparinized blood, which was to be tested for insulin, was injected into the jugular vein.
1:30	0.05 cc. of blood was taken from the tail for glucose determination ( <i>sample A</i> ). The animal was returned to the warm chamber.
1:45	0.05 cc. of blood was removed for glucose determination ( <i>sample B</i> ).
2:00	0.05 cc. of blood was removed for glucose determination ( <i>sample C</i> ).

For the blood glucose determination the micromethod of Haslewood and Strookman (1939) was employed. The difference between blood glucose values in samples A and C was taken as the measurement of insulin effect. The glucose value of sample B was not used in computing insulin effect, but having this reading fall in an intermediate position between A and C gave supportive information.

*EXPERIMENTAL DATA. Glucose tolerance in the ADH rat.* The blood sugar response to the feeding of glucose is shown in figure 1. The glucose tolerance test was carried out as described in the outline of procedure. Group I includes 15 ADH rats which were subjected to the test without an anesthetic. Group

II represents 17 similar animals which had been anesthetized with nembutal. The animals of group II were more severely diabetic than those of group I. This was due to the fact that as our technique improved, more of the severely diabetic rats survived during the period of alloxan administration. In spite of this increase in severity of the diabetes, the rats of group II, under nembutal, showed less rise in blood sugar over the 30-minute period in which they were studied than did the rats of group I which had no anesthetic for the same period. It is presumed that the rise in blood sugar in the unanesthetized animals was due in large part to the liberation of sympathin occasioned by the struggling during the procedure.

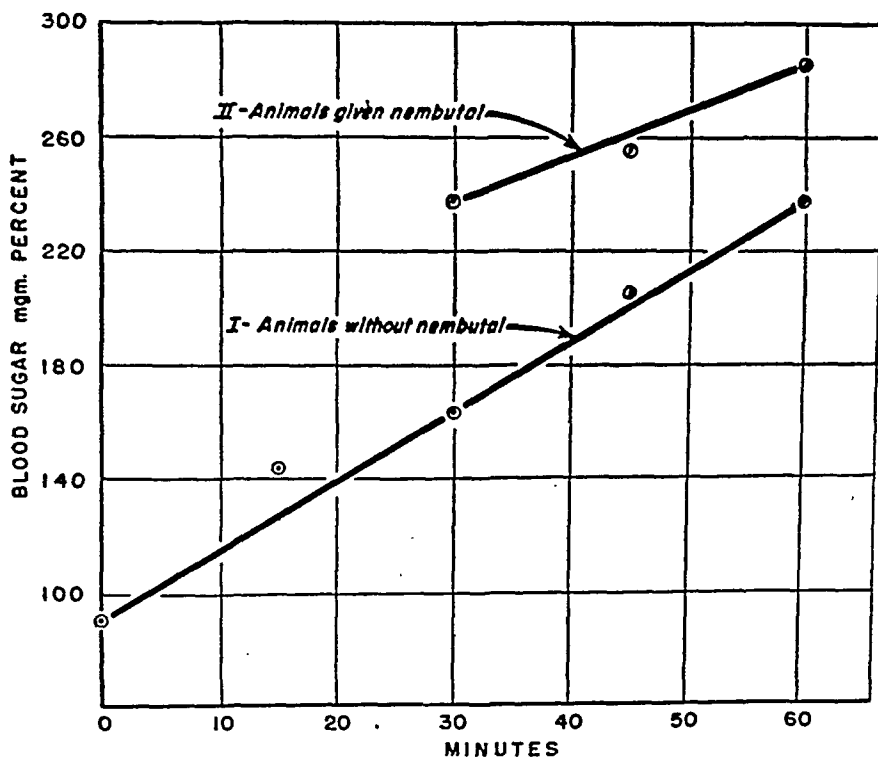


Fig. 1. Glucose tolerance curves in ADH rats. Curve I represents 15 animals which were not anesthetized during the test, and curve II, 17 animals which were anesthetized with nembutal.

*The response of the ADH rat to known amounts of insulin.* Two standard solutions of insulin in plasma were used, one containing 1.0 milli-unit per cc., and the other 0.5 milli-unit per cc. The response of the ADH rat to intravenous administration of graded doses of insulin is shown in table 1. The dose of insulin is expressed in milli-units per animal rather than per body weight of the animal. The rats were all approximately the same age and at the time of hypophysectomy weighed between 225 and 250 grams. Following hypophysectomy their weights decreased to about 200 grams.

The data on two groups of controls are shown in table 1. When no intravenous injection was given, the rise in blood sugar was +48 mgm. per cent. When plasma from rats fasted for 17 hours was given intravenously the rise in

blood sugar was essentially the same, namely +54 mgm. per cent. We have concluded, therefore, if there is any insulin in the blood of fasted animals, it

TABLE 1

*Response of ADH rat to intravenous administration of insulin*

NO. ANIMALS	INTRAVENOUS ADMINISTRATION		CHANGE IN BLOOD GLUCOSE IN 30-MINUTE PERIOD
	Insulin milli-units	Plasma	
		cc.	mgm. %
17	0	0	+48 $\pm$ 13*
19	0	1-2	+54 $\pm$ 28.3
13	0.125	0.25	-11 $\pm$ 4.8
16	0.25	0.50	-21 $\pm$ 9.6
17	0.50	1.00	-43 $\pm$ 11.0
17	1.00	1.00	-50 $\pm$ 24.8
8	2.00	2.00	-52 $\pm$ 11.4
9	5.00	1.00	-40 $\pm$ 21.0

\* Standard deviation.

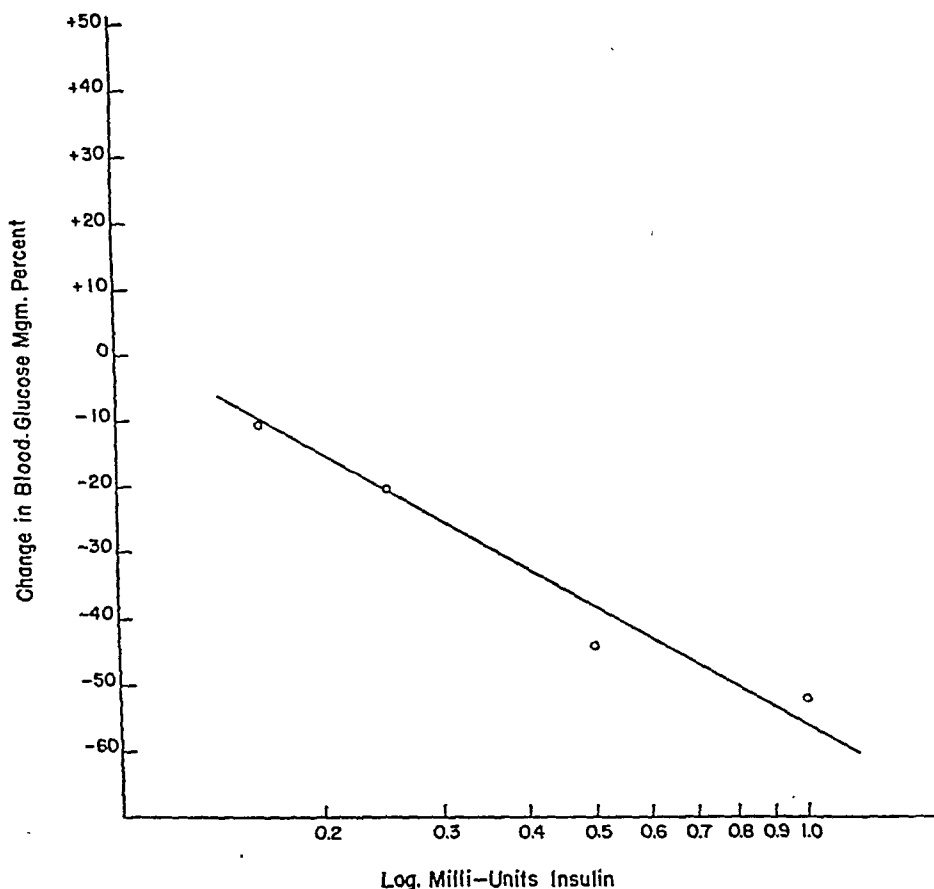


Fig. 2. The logarithm dose—arithmetic response relationship of the assay of insulin.

is not in a sufficient amount to be detected by this method. It will be noted that a dose of insulin of 0.125 milli-unit induces a significant fall in blood sugar.

If this value were to be expressed in milli-units of insulin per 100 grams of body weight (the ADH rat weighing about 200 grams), it would be approximately 0.062. The dose-response curve in figure 2 indicates a linear relationship between the arithmetic value for the change in blood sugar and the logarithm of the dose.

**DISCUSSION.** The rationale of using an adrenodemedullated hypophysectomized rat as a test animal for the assay of insulin is quite evident. Cannon, McIver and Bliss (1924) first showed that adrenalin is mobilized in response to insulin hypoglycemia; Houssay and Magenta (1925) were the first to demonstrate the hypersensitivity of the hypophysectomized animal to insulin.

There may be some question as to the reasons for rendering the animal diabetic by the administration of alloxan or of the giving a glucose meal. In order to avoid any discrepancies due to the secretion of endogenous insulin it was considered necessary to eliminate all insulin-secreting tissue. The question whether the absence of the beta cells of the islets makes the ADH rat more sensitive to insulin cannot be answered at the present time. The giving of a glucose meal was made a part of the assay procedure in order to have a blood sugar level which was rising rather than falling at the outset of the test. This eliminated the uncertainty of a fatal hypoglycemia which may occur in a fasted ADH rat whether it has been given insulin or not. In the practical working out of the test we found that a rise in blood sugar level over the 30-minute period which was studied, always occurred when no insulin was administered, and in all the cases where known amounts of insulin were given there was a fall in blood sugar in this same period.

#### SUMMARY

1. A method for the detection of minute amounts of insulin in blood is described.

2. The test animal used was an adrenodemedullated diabetic hypophysectomized (ADH) rat.

3. The glucose tolerance curve of the ADH rat showed less of a rise in blood sugar when the animals were tested under nembutal anesthesia.

4. A dose of insulin as low as 0.125 milli-unit (milli-unit = 0.001 unit) causes a significant fall in the blood sugar of an ADH rat weighing about 200 grams.

5. The dose-response curve shows a linear relationship between the arithmetic value of the change in blood sugar and the logarithm of the dose of insulin.

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# THE INHIBITORY EFFECT OF CARONAMIDE ON THE RENAL ELIMINATION OF PENICILLIN<sup>1</sup>

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It has been established that the renal elimination of penicillin is by both glomerular filtration and tubular excretion. These combine to account for the most seriously limiting pharmacodynamic property of penicillin: its very rapid renal elimination.

A number of measures have been evolved to prolong the retention of penicillin in the body. This has been accomplished either by delaying its absorption from an intramuscular depot or by inhibiting its excretion. The first method sacrifices intensity of effect for prolongation of a demonstrable blood level, since such a measure does not influence directly the excretion of penicillin.

The clearance of penicillin in both man and dog approximates renal plasma flow at all plasma concentrations that have been studied (1). Since in man the fraction of renal plasma flow that is filtered at the glomerulus is about 20 per cent, this means that some 80 per cent of the renal elimination of penicillin may be by tubular excretion. With the aid of high plasma concentrations of Diodrast<sup>2</sup> or PAH (p-aminohippurate) it has been possible to suppress the tubular excretion of penicillin on a "mass action" basis, since these adjuvants are excreted by the same tubular transport mechanism as is penicillin (2). This "mass action" principle has been applicable in therapy (3), but the large dosage of drug that must be administered intravenously to produce this effect limits its clinical usefulness from a practical standpoint.

Recently Beyer introduced an hypothesis for the competitive inhibition of the tubular transport mechanism that excretes penicillin, PAH, diodrast, etc., wherein the blocking agent was refractory to tubular excretion (4). It appeared that the renal elimination of the compound studied was essentially by glomerular filtration and that it did not alter other renal transport systems (5) or impair the vitality of the cells or tissues as a whole (6). The hypothesis was set forth in the article referred to above (4). The differentiation of this mode of inhibition from others has been discussed in more detail in the publication by Beyer, Russo, Patch, and Tillson (5).

The compound, 4'-carboxyphenylmethanesulfonanilide, was described by

<sup>1</sup> Presented at the Conference on Antibiotic Research held at Washington, D. C. on January 31-February 1, 1947, under the auspices of the Antibiotic Study Section of the National Institute of Health.

<sup>2</sup> "Diodrast" is the trademark of the Winthrop Chemical Company, Inc. for Diiodopyridone acetic acid diethanolamine.

Sprague, Ziegler, Miller and Cragoe (7) and has been referred to herein as caronamide.

The purpose of this research has been to evaluate the inhibitory effect of caronamide on the renal elimination of penicillin.

*Structural relationship of caronamide to penicillin G.* It was proposed that to be effective a compound should have an affinity for the particular enzyme system that characterizes the selectivity of the overall tubular mechanism for penicillin excretion. Moreover it should be sufficiently different to be essentially refractory to excretion by the tubules.

Superficially there is a similarity of the structural formula of caronamide and penicillin G; however the evaluation of other compounds for this purpose has shown that these pharmacologic properties of the chemical agent could not have been predicted. In a subsequent section of this report it will be shown that caronamide inhibits the renal elimination of other types of penicillin such as K, F and X to as great an extent as of penicillin G. Also, it can be shown to inhibit the tubular excretion of PAH and other compounds totally unrelated structurally to caronamide (5).

*The effect of caronamide on the bacteriostatic activity of sulfathiazole* was evaluated since in clinical practice penicillin and sulfonamide therapy occasionally are combined. A dilute suspension of *Escherichia coli* was used as seed in tubes containing a synthetic medium to which were added a constant amount of caronamide and varying amounts of sulfathiazole. After incubation, growth was recorded in terms of turbidimetric density estimations. Although results of these experiments showed that caronamide had a slight antagonistic effect on the bacteriostatic activity of sulfathiazole, calculations demonstrated that it would be necessary to use 1000 mgm. of caronamide to antagonize the effect of 1 mgm. of sulfathiazole. This same amount of sulfathiazole (1 mgm.) was antagonized by only 0.3 mgm. of p-aminobenzoate under these same conditions. The inhibition of sulfonamide activity that appears to be caused by caronamide therefore is believed to be of little practical significance.

Bacteriostatic tests of caronamide against laboratory strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridium welchii*, *Leuconostoc mesenteroides* and *Brucella abortus* were performed. With the exception of *C. welchii*, all the organisms were able to grow at a concentration of 150 mgm. of caronamide/100 cc. of culture medium. *C. welchii* grew well at a concentration of 75 mgm. of drug/100 cc. but was inhibited at 150 mgm./100 cc.

*The effect of caronamide upon the estimation of penicillin in assays* was determined since this was a potential source of error in the work. Florey cup-plate assays, using *Staphylococcus aureus* 209 P, of plasma, urine, and buffered penicillin solutions with and without caronamide were performed. At concentrations of 30 mgm./100 cc. of plasma and 150 mgm. in 100 cc. of urine or buffer, the drug had no demonstrable effect upon the penicillin values that were obtained.

*The renal clearance of amorphous penicillin as influenced by caronamide.* Throughout these experiments amorphous penicillin obtained in the open market was used except where specifically indicated in the test. Assays were performed by the Florey cup-plate method in the case of solutions of penicillin, urine, and citrated plasma. Reasonably aseptic precautions were observed in the collection of blood and urine. The clearances were performed on carefully trained dogs taught to lie loosely restrained on a comfortably padded table. Sedation of the animals was not required or employed in these experiments. A detailed protocol is included in the first of the experiments in table 1.

Essentially, these experiments were composed of a control phase that included duplicate penicillin clearances and a second phase wherein the effect of the drug on penicillin clearance, plasma concentration, etc. was determined. The design of the experiments included a number of desirable cross-checks. These are illustrated in the interpretation of table 1 which summarizes three such determinations.

In the first experiment, table 1, it may be seen that caronamide, at a maintenance infusion of 30 mgm./kgm./hr., suppressed the renal clearance of penicillin to the point that it approximated glomerular filtration rate (creatinine clearance). This may be taken to mean that the excretion of penicillin by the tubules was completely inhibited and glomerular filtration remained its only mode of renal elimination; therefore, the ratio of the penicillin clearance to creatinine clearance (clearance ratio) decreased to or below 1.0. The maintenance of a uniform urine flow and a reasonably constant creatinine clearance attest to the lack of acute toxicity of the drug and the satisfactory character of the test.

The selection of a priming and maintenance dosage of caronamide of 25 mgm./kgm. and 30 mgm./kgm./hr., respectively, had been made in earlier research on a series of compounds as one most uniformly giving a plasma concentration of 10 mgm./100 cc. Under the conditions of this experiment PAH had little or no effect on penicillin clearance, numerically or as represented in the clearance ratio. In the evaluation of new compounds clearance ratio data are more reliable frequently than either the penicillin clearance or increase in plasma concentration of the antibiotic agent. As the elimination of penicillin by the tubules is suppressed its clearance decreases to approach that for creatinine (glomerular filtration rate) as a theoretical limit if there is no plasma binding or reabsorption of the antibiotic agent. It will be found in some of these data that the clearance ratio for penicillin becomes less than 1.0 which may be taken to substantiate the claim that some penicillin is bound on plasma proteins (8), though a slight back diffusion of the agent through the tubules is not ruled out. Usually, the initial penicillin and creatinine clearances are higher than the second control value. These differences are of a technical nature and may reflect excessive hydration since the clearance ratio remains reasonably constant or is decreased slightly under these conditions.

The second and third experiments in table 1 are repetitions of the first except

TABLE 1

*The design of the experiments demonstrating the effect of caronamide on the renal clearance, plasma concentration, and clearance ratio of penicillin. Each experiment was performed at half the dosage of caronamide used in the preceding trial*

PERIOD	PENICILLIN		CREATININE CLEARANCE	CLEARANCE RATIO	URINE FLOW
	Plasma conc.	Clearance			
Dog A, wt. 17.7 kgm.					
hr.:min.	u/cc.	cc./min.	cc./min.		cc./min.
9:30	700 cc. H <sub>2</sub> O p.o.				
10:30	400 cc. H <sub>2</sub> O p.o.				
10:45	Began i.v. infusion of 156 units penicillin/min. in 5% glucose soln. at 3 cc./min.				
10:50	Penicillin priming dose, 15,200 units, i.v.				
10:53	3.0 grams creatinine, s.c.				
Control phase, penicillin alone					
11:20	0.68	278.1	75.2	3.70	3.1
11:30	0.58	212.8	63.6	3.33	3.3
11:37	Infusion changed to 30 mgm./kgm./hr. Caronamide plus 137 units penicillin/min. in 5% glucose soln. at 3 cc./min.				
11:40	Caronamide priming dose, 25 mgm./kgm., i.v.				
Drug phase, penicillin plus caronamide					
12:15	0.79	51.9	60.3	0.86	3.5
12:25	0.83	57.2	59.5	0.96	3.6
Dog B, wt. 16.4 kgm.					
	Penicillin: Priming dose 15,200 units, i.v. Maintenance dose 156 u./min. at 3 cc./min., i.v.				
Control 1	0.53	315.4	70.1	4.55	1.10
Control 2	0.48	227.7	58.6	3.85	0.95
	Penicillin: Maintenance dose 130.5 u./cc., at 3 cc./min., i.v. Drug: Priming dose 12.5 mgm./kgm., i.v. Maintenance dose 15 mgm./kgm./hr.				
Drug 1	0.54	83.0	62.6	1.33	1.20
Drug 2	0.60	68.3	55.6	1.23	1.10
Dog C, wt. 24.0 kgm.					
	Penicillin: Priming dose 17,000 units, i.v. Maintenance dose 145.5 u./min. at 3 cc./min., i.v.				
Control 1	0.50	364.0	77.6	4.76	5.6
Control 2	0.50	282.2	76.0	3.70	5.5
	Penicillin: Maintenance dose 136.5 units/min. at 3 cc./min., i.v. Drug: Priming dose 6.25 mgm./kgm., i.v. Maintenance dose 7.5 mgm./kgm./hr.				
Drug 1	0.58	122.4	66.4	1.85	3.5
Drug 2	0.62	122.5	71.5	1.72	3.5

that the dosage of caronamide in each instance is successively  $\frac{1}{2}$  that in the preceding test. From these data it is apparent that by any or all criteria the compound effectively inhibits the renal tubular excretion of penicillin. This effect is related to dosage or plasma concentration of the drug. Repeated trials wherein the determinable plasma concentration of other more or less related compounds have been obtained under these conditions indicate that the priming and maintenance dose of 12.5 mgm./kgm. and 15 mgm./kgm./hr. may be expected to give a plasma level of about 6 mgm./100 cc. The priming dosage of 6.25 mgm./kgm. and maintenance dose of 7.5 mgm./kgm./hr. should yield a plasma concentration of about 2 to 3 mgm./100 cc. Preliminary analytical data on the plasma concentrations of caronamide, though unreliable as yet, bear out the above figures.

These data indicate that caronamide very effectively suppresses the renal clearance of penicillin. Depending on the dosage of the drug, excretion of penicillin by the tubules can be completely or partially blocked.

*The effect of caronamide on the renal clearance of various types or fractions of penicillin.* These experiments seemed indicated for two reasons. It has been thought that a considerable difference exists between the excretion of the various penicillin fractions, although to our knowledge no data defining their clearances have been published.

Various crystalline penicillins were obtained through the courtesy of other investigators. The renal clearances of these agents together with the effect of caronamide thereon were studied by the same method as was outlined in the previous section, but using triplicate clearances. The results of these studies are represented by experiments on the various types of penicillin in tables 2, 3, 4 and 5.

The clearance values for the various types of penicillin are of considerable interest themselves. Although they behave quite differently in some respects, penicillin G, K, F and X have essentially similar renal clearances at the plasma levels used in these experiments. We were able to use the same experimental set up for penicillins F, G, and X, but two trials had to be sacrificed before a priming and maintenance dosage of penicillin K was obtained. It was necessary to use approximately a 3-fold increase in priming dose and a 6 to 8-fold increase in the maintenance dose of penicillin K in order to offset its rapid inactivation and at the same time give suitable plasma and urine values for our experiments. At the standard priming and maintenance dose no penicillin K was found in any of the blood samples and a determinable amount was found in only the first urine sample. These findings substantiate what is known regarding its rapid inactivation (9).

In these experiments caronamide appears to inhibit the renal elimination of all four penicillins to the same full extent of their tubular excretion. It is important to know this even though penicillin G predominates in the present commercial products.

From our limited information on crystalline penicillins in comparison with our greater use of the amorphous products two points seem worth mentioning.

Except for penicillin K the variation in the control crystalline penicillin clearances was less than we were accustomed to observe when the amorphous material was used, and as yet none of these clearances has been greater than 300 cc./min., as frequently happens for amorphous material. Also, we have not observed as frequently penicillin clearance ratios below 0.77 following administration of caronamide with amorphous penicillin as occur in these data.

TABLE 2

*The effect of caronamide on the renal clearance of crystalline sodium penicillin F*  
Dog A, wt. 18.0 gm.

10 MINUTE CLEAR- ANCE PERIODS	PENICILLIN F*		CREATININE CLEARANCE	CLEARANCE RATIO	URINE FLOW
	Plasma conc.	Clearance			
	u/cc.	cc./min.	cc./min.		cc./min.
	Penicillin: Priming dose 23,800 units, i.v. Maintenance infusion 121 units/min. in 5% glucose soln. at 3 cc./min., i.v.				
Control 1	0.72	223.4	48.5	4.56	0.70
Control 2	0.53	229.6	74.5	3.13	1.60
Control 3	0.63	215.4	79.2	2.70	2.35
	Penicillin: Maintenance infusion 108 units/min. as above. Caronamide: Priming dose 25 mgm./kgm., i.v. Maintenance infusion 30 mgm./kgm./hr.				
Drug 1	0.69	61.2	60.9	1.00	3.32
Drug 2	0.86	50.8	66.1	0.77	3.52
Drug 3	0.94	51.6	71.9	0.72	3.70

\* The properties of sample NIH-F 1156 were reported to us as follows:

Properties	Sample
Analysis for CHN and double bond.....	Satisfactory for $C_{14}H_{19}O_4NSN_a$ .
Biological assays (based on penicillin G standard)	
<i>S. aureus</i> .....	1625 units/mgm.
<i>B. subtilis</i> .....	1075 units/mgm.
Craig Distribution Curve.....	"free of X and K types, possibly a few per cent G"

*The effect of orally administered caronamide on the blood level and renal clearance of penicillin administered continuously by venoclysis.* These experiments were designed to evaluate the efficacy and duration of effect of caronamide administered orally. It was necessary to do this indirectly since a direct assay for the compound was not available. However, the efficacy and duration of drug action could be measured by its effect on penicillin clearance and clearance ratio when the antibiotic agent was administered continuously at a constant rate. The design of the experiments is given in the protocol of table 6.

In the initial experiments the drug was compared to other agents at an oral dosage of 150 mgm./kgm. While this dosage was well tolerated in all respects

by the animals, it proved excessive since the excretion of penicillin by the tubules was suppressed completely, the clearance ratio being equal to or below 1.0. An oral dosage of 25 mgm./kgm. behaved similarly but was less effective in decreasing the clearance of penicillin.

At a dosage of 50 mgm./kgm. the duration of a determinable effect of caronamide was at least four hours as judged by the penicillin/creatinine clearance ratio, penicillin clearance, and plasma concentration, table 5. The effect of the

TABLE 3

*The effect of caronamide on the renal clearance of crystalline sodium penicillin G*  
Dog B, wt. 18.0 kgm.

10 MINUTE CLEAR- ANCE PERIODS	PENICILLIN G*		CREATININE CLEARANCE	CLEARANCE RATIO	URINE FLOW
	Plasma conc.	Clearance			
	u/cc.	cc./min.	cc./min.	cc./min.	
	Penicillin: Priming dose 10,900 units, i.v. Maintenance infusion 108 units/min. in 5% glucose soln. at 3 cc./min., i.v.				
Control 1	0.51	291.1	94.3	3.13	0.65
Control 2	0.46	260.7	90.1	2.86	1.25
Control 3	0.37	227.9	82.8	2.78	1.80
	Penicillin: Maintenance infusion 70 units/min., i.v. as above. Caronamide: Priming dose 25 mgm./kgm., i.v. Maintenance infusion 30 mgm./kgm./hr., i.v.				
Drug 1	0.59	59.7	84.6	0.70	1.25
Drug 2	0.70	53.8	89.4	0.60	1.45
Drug 3	0.76	47.3	84.8	0.56	1.80

\* The properties of sample NIH-G 1156 compared to the National Master Standard for penicillin G are:

Property	Sample	Standard
Specific Rotation ( $\alpha$ )D <sup>25</sup> .....	+305.6	+304.7
S. aureus assay, units/mgm.....	1707	1667
Crystallographic Refractive Indices:		
$\alpha$ .....	1.550	1.550
$\beta$ .....	1.609	1.609
$\gamma$ .....	1.620	1.620
Purity by chromatographic analysis.....	99.5	100

compound on the excretion of penicillin by the tubules was completely gone at the time of the duplicate clearances six hours following its oral administration.

From these experiments certain tentative impressions that were of value in the design of work to be described in later sections of this report were drawn. The dosage of 50 mgm./kgm. seemed to be quite feasible; its demonstrable effects persisted for at least four hours; and the drug effect was completely reversible.

*Experiments wherein penicillin and varying amounts of caronamide were administered by venoclysis for 36 hours.* The purpose of these experiments was

TABLE 4

*The effect of caronamide on the renal clearance of crystalline sodium penicillin K*  
Dog E, wt. 16.7 kgm.

10 MINUTE CLEAR- ANCE PERIODS	PENICILLIN K*		CREATININE CLEARANCE	CLEARANCE RATIO	URINE FLOW
	Plasma conc.	Clearance			
	u/cc.	cc./min.	cc./min.		cc./min.
	Penicillin: Priming dose 51,300 units, i.v. Maintenance infusion 912 units/min. in 5% glucose soln. at 3 cc./min., i.v.				
Control 1	0.65	202.4	67.1	3.03	5.8
Control 2	0.68	160.6	68.7	2.33	5.4
Control 3	0.55	233.9	75.6	3.13	5.8
	Penicillin: Maintenance infusion 816 units/min., i.v. as above. Caronamide: Priming dose 25 mgm./kgm., i.v. Maintenance infusion 25 mgm./kgm./hr., i.v.				
Drug 1	0.69		65.6		1.0
Drug 2	0.81	49.5	65.6	0.75	2.3
Drug 3	0.83	38.1	51.4	0.74	2.9

\* The properties of sample NIH-K 1156 were reported to us as follows:

Properties	Sample
CNH Analyses.....	Satisfactory for $C_{16}H_{25}O_4N_2SN_3$
Optical rotation $(\alpha)D^{25}$ .....	+279°
Biological assays (based on penicillin G standard)	
E. coli turbidimetric assay.....	210 u./mgm.
S. aureus turbidimetric assay.....	2620 u./mgm.
S. aureus Oxford plate assay.....	2190 u./mgm.

TABLE 5

*The effect of caronamide on the renal clearance of crystalline sodium penicillin X*  
Dog D, wt. 24.3 kgm.

10 MINUTE CLEAR- ANCE PERIODS	PENICILLIN X*		CREATININE CLEARANCE	CLEARANCE RATIO	URINE FLOW
	Plasma conc.	Clearance			
	u/cc.	cc./min.	cc./min.	cc./min.	
	Penicillin: Priming dose 19,400 units, i.v. Maintenance infusion 136.5 units/min. in 5% glucose soln. at 3 cc./min.				
Control 1	0.59	205.0	125.6	1.64	7.7
Control 2	0.55	224.4	114.6	1.96	6.3
Control 3	0.44	222.6	102.1	2.17	6.3
	Penicillin: Maintenance infusion 128.2 units/min. i.v., as above. Caronamide: Priming dose 25 mgm./kgm., i.v. Maintenance infusion 30 mgm./kgm./hr., i.v.				
Drug 1	0.67	65.0	89.7	0.72	5.0
Drug 2	0.80	60.4	89.4	0.68	4.6
Drug 3	0.81	61.9	83.3	0.74	3.6

\* The properties of sample NIH-X 1156 were reported to us as follows:

Properties	Sample
Potency assay (based on penicillin G standard).....	970 u./mgm.
Penicillin X content by ultra violet absorption data at 2800 A°...	96.5%

to determine the effect of different dosages of caronamide on the blood level, renal clearance and clearance ratio of penicillin administered at a constant rate by venoclysis.

TABLE 6

*The effect of 50 mgm. caronamide per kgm. administered orally on the renal clearance and blood level of penicillin infused by venoclysis*

PERIOD	PENICILLIN		CREATININE CLEARANCE	CLEARANCE RATIO	URINE FLOW
	Plasma conc.	Clearance			
Dog D, wt. 24.1 kgm.					
hr.:min.	u/cc.	cc./min.	cc./min.		cc./min.
-2:15	700 cc. H <sub>2</sub> O p.o.				
-1:20	400 cc. H <sub>2</sub> O p.o.				
-1:00	Began i.v. infusion of 125 units penicillin/min. in 5% glucose soln. at 3 cc./min.				
-0:58	Penicillin priming dose, 10,000 units, i.v.				
-0:56	3.0 grams creatinine s.c.				
Triplicate control penicillin clearance					
-0:30	0.68	292.1	112.4	2.63	9.5
-0:20	0.54	315.9	109.6	2.86	8.2
-0:10	0.50	341.0	104.8	3.23	5.5
0:00	Caronamide 50 mgm./kgm. in 50 cc. aqueous soln., p.o.				
1:00	200 cc. H <sub>2</sub> O p.o.				
1:30	Penicillin priming dose 7,500 units, i.v. Maintenance dose 125 units/min. in 5% glucose soln. at 3 cc./min.				
1:35	1.5 grams creatinine, s.c.				
2:00	0.92	133.3	102.6	1.30	1.8
2:10	0.83	141.4	101.0	1.41	3.9
3:00	400 cc. H <sub>2</sub> O p.o.				
3:30	Penicillin priming dose 7,500 units, i.v. Maintenance dose 125 units/min. in 5% glucose soln. at 3 cc./min.				
3:35	1.5 grams creatinine, s.c.				
4:00	0.68	240.3	100.2	2.38	7.2
4:10	0.61	240.3	100.1	2.38	6.4
5:00	400 cc. H <sub>2</sub> O p.o.				
5:30	Penicillin priming dose 7,500 units, i.v. Maintenance dose 125 units/min. in 5% glucose soln. at 3 cc./min.				
5:35	1.3 grams creatinine, s.c.				
6:00	0.50	346.6	104.0	3.33	8.1
6:10	0.43	292.6	96.6	3.13	7.4

Female dogs were weighed, given water by stomach tube, and anesthetized with sodium pentobarbital. The bladder was catheterized, and the catheter was secured aseptically through a suprapubic approach and ligation of urethra

and tube. A vein on the foreleg was exposed for the taking of blood samples. Penicillin alone or with caronamide was infused at a constant rate of 2 cc./min. with the aid of an electrically driven constant speed infusion pump. In addition to penicillin and caronamide the solution contained 0.8 per cent NaCl and 0.5 per cent glucose. All solutions were sterilized and aseptic precautions were observed throughout the experiments. These included the collection of urine with the aid of an instrument previously described in order to minimize bacterial contamination (1a). Precautions to avoid deterioration of the penicillin in the infusion solution, blood, and urine were taken. Creatinine was administered subcutaneously at intervals throughout the experiments so that simultaneous creatinine and penicillin clearances could be determined. Additional barbiturate solution was administered as required.

At the beginning of an experiment an initial intravenous injection of 7,500 units of sodium penicillin was made and the constant venoclysis of approximately 125 units of penicillin per minute was begun. This infusion was continued as a control phase for 8 to 12 hours. At 2-hour intervals throughout the last portion of the control phase three 15-minute clearances were performed.

At the end of the control phase a single intravenous dose of caronamide that was equivalent to the dose in mgm./kgm./hr. administered continuously during the first 8 hour drug phase was injected. In the example given in figure 1 the priming dose was 30 mgm./kgm. and the maintenance infusion was 30 mgm./kgm./hr. Here again, three 15-minute penicillin and creatinine clearances were determined at 2-hour intervals during the last 6 hours of this phase.

In the second drug phase the priming and maintenance dosages were doubled. The duration of this phase was 8 hours and the triplicate clearance determinations were performed at 2-hour intervals throughout the last 6 hours of the period.

Immediately after the last clearance of the second drug phase the infusion of caronamide was discontinued, the rate of penicillin infusion remaining essentially the same. After the first four hours of this second control phase, clearance determinations were made at 2-hour intervals for the remainder of this 10-hour period. At the termination of the experiment the dogs were reweighed and autopsied, and sections were taken for histologic examination.

Several different dosages were used in the various experiments and in still other tests the protocol was duplicated except for the omission of caronamide. These latter experiments served as controls on the overall design of this phase of the problem.

The experiment summarized in figure 1 serves to illustrate most of the conclusions that may be derived from this part of the work. In the initial control phase it may be seen that the plasma concentration of penicillin was stabilized at about 0.5 u./cc. There was some variation in the renal clearance of the antibiotic agent; however the creatinine clearance, although normal, varied sufficiently so that the clearance ratio was fairly stable at about 3.6 to 2.7, despite the alteration in penicillin clearance. Frequently, in these experiments, creatinine clearances were higher than is seen in unanesthetized dogs of the same

weight. Also, in the control experiments wherein no caronamide was administered, tremendous simultaneous variations in both penicillin and creatinine clearances occasionally were observed.

From the rising plasma concentration of penicillin in the first drug phase it would appear that the effect of caronamide on penicillin blood level had not

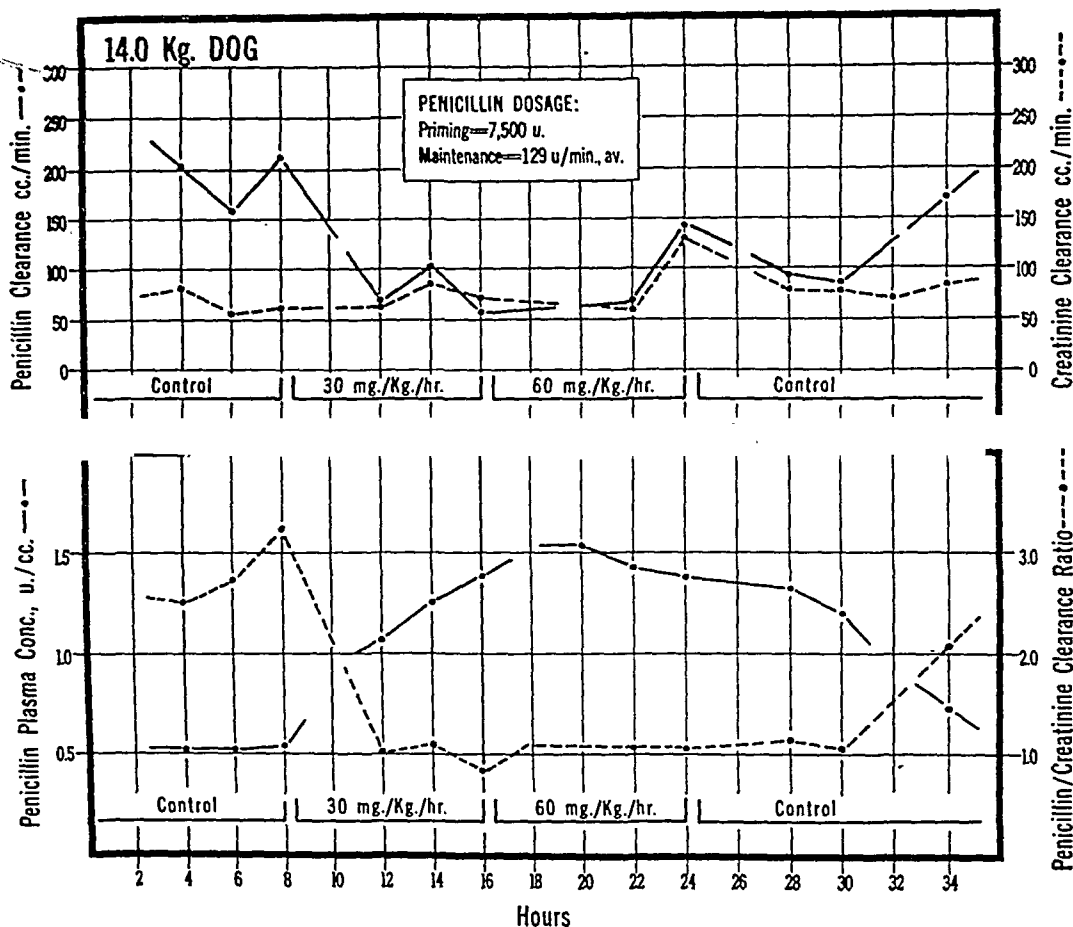


Fig. 1. Results of an experiment wherein penicillin and caronamide were administered by venoclysis. The initial injection of penicillin was 7,500 units followed by an infusion of 129 units/minute throughout the 34 hours. From the eighth to the sixteenth hour caronamide also was infused at a rate of 30 mgm./kgm./hr. From the sixteenth to the twenty-fourth hour the caronamide dosage was 60 mgm./kgm./hr. In the upper graph penicillin and creatinine clearance are plotted, and in the lower graph penicillin plasma concentration and clearance ratio are given. The time ordinate is the same for both graphs.

yet become stabilized even though tubular excretion of the drug was practically or completely abolished at all three clearances, as evidenced by a penicillin/creatinine clearance ratio of about 1.0.

At the end of the fourth hour of the second drug phase the plasma concentration of penicillin had reached its peak and was stabilized.

This was at a value about 3 times the initial control plasma level of penicillin.

This three-fold increase in penicillin drug level was all that could be expected in this experiment. In the initial phase the penicillin/creatinine clearance ratio was 3.6 to 2.7. Thus if tubular excretion was completely suppressed so that filtration represented the total excretion of penicillin (clearance ratio—1.0) its reduction in excretion would not amount to more than three-fold. Since the renal clearance of the antibiotic agent was suppressed to approximately creatinine clearance in the first drug phase it appears that the dosage of 60 mgm./kgm./hr. was excessive and was without greater effect than would have been obtained if the first drug period had been extended until the penicillin plasma concentration had equilibrated. The doubling of the creatinine clearance at the end of the second drug phase probably was responsible for the fact that the highest penicillin blood level was not entirely sustained. This was not an effect of caronamide on creatinine clearance, for we have seen as great fluctuations in glomerular filtration rate in similar experiments wherein none of the drug was administered.

Following the termination of the second drug phase several hours were required for the urinary elimination of the excessive amount of drug before the plasma concentration of penicillin began to fall. The fall in penicillin blood level almost to normal at the end of the experiment together with the return of penicillin clearance to within the limits of the variation seen in the initial control phase are indicative of the reversibility of the effect of caronamide on penicillin excretion. From these experiments it appears that the least amount of caronamide sufficient to maintain a penicillin/creatinine clearance ratio of 1.0 or less should be adequate to bring about the maximal penicillin blood level response. The amount of caronamide needed to maintain a penicillin/creatinine clearance ratio of 1.0 is dependent on glomerular filtration rate; that is, the greater the creatinine clearance the greater the dosage of caronamide needed to produce a maximal effect. In all our experiments cessation of caronamide infusion has been followed by a return of penicillin blood levels and renal clearance to, or approximately to, control values.

The rapidity of this recovery seems to be dependent on the rate of elimination of caronamide from the body.

*Concerning the blood level response to the repetitive oral administration of penicillin alone, and with the coadministration of caronamide.* These experiments were run in duplicate, the dogs usually being litter mates or of the same species and of uniform weight and age. They were given extra rations of meat and milk the night before the experiment. The next morning they were fed 5 per cent glucose in 200 cc. of milk about 3 hours before the experiments were begun. Thereafter they were placed in metabolism cages, allowed water ad libitum but food was withheld.

At the beginning of the experiment each dog was given 150,000 units of penicillin in 50 cc. of tap water by stomach tube. Thereafter at 4-hour intervals for the remainder of the test they were given 100,000 units in water by stomach tube. Citrated blood samples were taken at 2 and 4 or 1, 2, 3 and 4 hour intervals following each oral dose of penicillin, or caronamide and penicillin. At

intervals of 6 to 10 hours during the experiments the dogs were given a 5 per cent solution of glucose in 250 cc. of milk. This was administered immediately following withdrawal of a blood sample so that the feeding would be at least one hour following a dose of penicillin and drug, and an hour before the next blood sample was taken.

After a 16-hour control phase wherein penicillin was administered alone, each dog was given 60 mgm. of caronamide/kgm. of body weight in solution with the customary 100,000 units of penicillin by stomach tube. At 4-hour

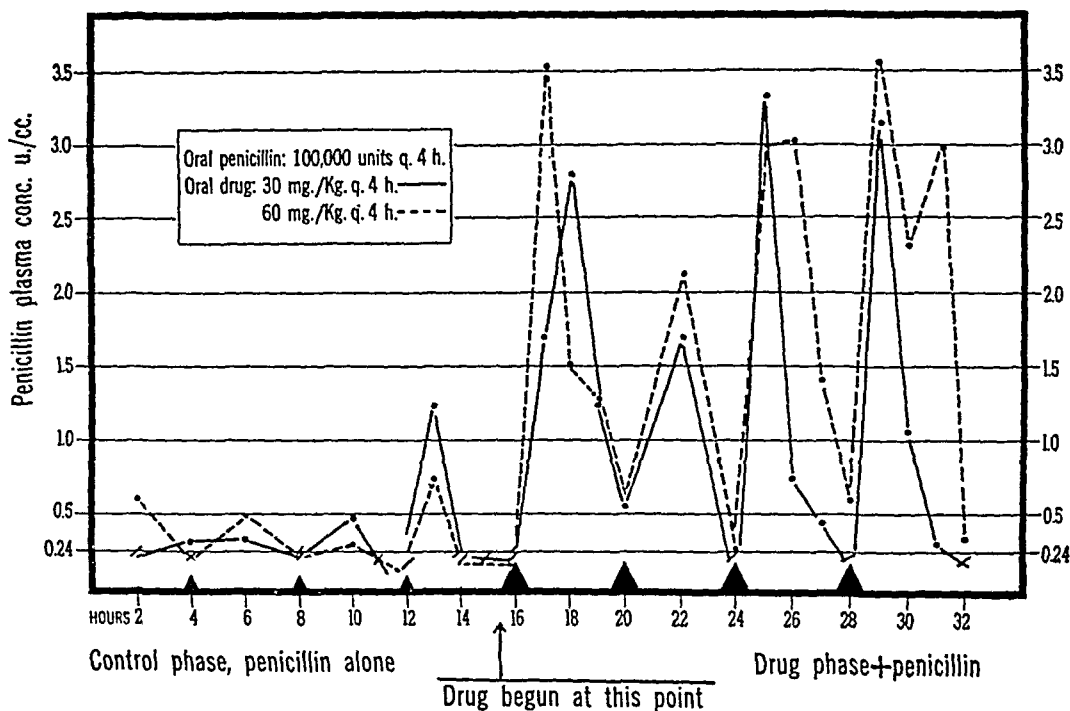


Fig. 2. The blood level response to the repetitive oral administration of penicillin alone, and with the coadministration of caronamide. Except for the initial 150,000 unit dose, 100,000 units of penicillin were administered every four hours throughout the experiment. After a sixteen hour control period each dog was administered 60 mgm. of caronamide/kgm. Thereafter one dog received 60 mgm./kgm. and the other was given 30 mgm./kgm. every four hours with penicillin.

intervals for the rest of the experiment one dog received a dose of 60 mgm./kgm., and the other was given 30 mgm. of caronamide/kgm. together with penicillin. The experiments usually were terminated after a duration of 32 hours.

The results of these experiments may be illustrated by the curves in figure 2. Here it may be seen that except for the one-(and usually two-) hour readings the values for penicillin blood level in the control phase were less than 0.24 u./cc., the lower limit of our Florey cup-plate assay method.

Following the coadministration of penicillin and caronamide the peak antibiotic blood levels were increased  $2\frac{1}{2}$  to 3-fold and there were only three 4-hour blood levels that were below the limits of the assay.

While the larger dosage of caronamide uniformly produced the greater penicillin blood level response, the greatest difference between the two dosages was in the duration of effect. It is important to point out that there has been no cumulative effect of the drug at either dosage in these and similar experiments.

It is outside the province of this paper to discuss the relationship of these results to therapeutic intensity. However, Verwey and Miller have studied the relative therapeutic effect of penicillin alone and with caronamide on pneumococcal and typhoid infections in mice (10). Their results have been expressed in terms of the dose of penicillin alone and with caronamide that would protect 50 per cent of the mice. These doses have been correlated with the height and duration of the plasma concentrations of the antibiotic agent. As might be expected, their experiments indicated that a considerable therapeutic advantage was obtained when caronamide was used as an adjunct to penicillin therapy.

#### SUMMARY

Caronamide decreased the tubular excretion and increased the plasma concentration of penicillin G, K, F and X. Its mode of action is to halt the tubular transport mechanism for penicillin excretion. This effect was reversible and the function of the system returned to normal after administration of the compound was stopped. It was effective when administered orally or parenterally and when penicillin was administered by the same or a different route.

The essential points in this report have been substantiated clinically (11).

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# CHANGES IN CERTAIN PHOSPHORUS AND CARBOHYDRATE CONSTITUENTS OF THE TISSUES OF RABBITS IN GRAVITY SHOCK

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Gravity shock may be produced in the rabbit by suspending the animal in a vertical position (1). It has been shown (2) that the composition of the blood of animals so treated undergoes a number of changes. In arterial blood the concentrations of lactate, pyruvate, and inorganic phosphate increase, while a decrease occurs in pH and in the concentrations of bicarbonate and glucose. The arterio-venous O<sub>2</sub> difference is also increased.

It has been suggested that the vertical suspension of the animal brings about a reduction of venous return and cardiac output, and that this circulatory deficiency leads to tissue hypoxia. Under these conditions the aerobic phase of tissue metabolism is depressed, and as a result, accumulation of lactate, pyruvate, and inorganic phosphate occurs in the tissues and the blood.

The evidence seemed to indicate that certain phases of carbohydrate and phosphorus metabolism are disturbed in gravity shock. In order to obtain more direct data on the nature and extent of these disturbances, a number of tissues were analyzed for appropriate phosphorus fractions, lactate, and glycogen. The results obtained, and their implication in the problem of shock are treated in this paper.

**METHODS.** A general description of the experimental procedure has already been given (2). The animals used in this study received only water for the 18-24 hours preceeding the experiment. During the production of shock, care was taken to minimize muscular exertion. In the final series of experiments, intensification of shock was achieved by first maintaining the animal at an angle of 45-60° until hyperpnea, tachycardia, and loss of exteroception were evident. This treatment was followed by a second stage in which the animal was lowered to an angle of about 20° and kept in that position until the desired state of shock was reached. The average time of suspension was 1 hour.

The following determinations were carried out.

<i>Determinations</i>	<i>Samples</i>
Hematocrit, pH, inorganic phosphate, acid soluble organic phosphate, and total phosphate	Whole blood
Inorganic phosphate, and acid soluble organic phosphate	Plasma

<sup>1</sup> The data of this paper are taken from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Physiology and Biochemistry, Rutgers University, New Brunswick, New Jersey.

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<i>Determinations</i>	<i>Samples</i>
Inorganic phosphate (IP), creatine phosphate (CP), adenosine pyrophosphate (APP), residual phosphate (RP), total acid soluble organic phosphate, and total phosphate	Gastrocnemius muscle, liver, kidney, duodenum
Lactate, and water	Gastrocnemius muscle
Glycogen	Gastrocnemius muscle, liver

The sampling of blood and determinations of hematocrit were carried out in the manner previously described (2).

Analyses of acid soluble phosphates of whole blood and plasma were performed according to the method of Fiske and Subbarow as modified by Guest and Rapoport (3), with the further substitution of amidol as the reducing agent (4). Whole blood total phosphate was determined as inorganic phosphate in a sample which had been wet ashed and neutralized. Acid insoluble phosphate represents the difference between acid soluble phosphate and total phosphate.

Samples of gastrocnemius muscle, duodenum, liver, and kidney, in that order, were obtained immediately after injection of a lethal dose of pentothal sodium (65 mgm./kgm.) into the heart. Effort was made to secure representative samples, to standardize the procedure, and to minimize handling. A portion (4 grams) of each tissue was dropped into a mixture of ether-CO<sub>2</sub> snow, and a second portion (1 gram) was placed in a corked tared flask. Each frozen tissue was then pulverized in a chilled mortar and portions of the powdered residue were transferred to tared flasks or centrifuge tubes kept in CO<sub>2</sub> snow. The samples were quickly weighed, taking care to remove external ice or moisture before final balancing, and again returned to the CO<sub>2</sub> snow. From this point, the determinations of lactic acid in muscle, and glycogen in muscle and liver were carried out as recommended by Blatherwick et al. (5).

Analyses of inorganic phosphate, creatine phosphate, adenosine pyrophosphate, and residual phosphate in tissue were performed, except for slight modifications, according to the method of Furchgott and Shorr (6). In the modified procedure, the strychnine phosphomolybdate precipitates were dissolved in 1 per cent NaOH, transferred to 30 ml. Kjeldahl flasks, and slowly digested according to the method of Guest and Rapoport (3). The phosphorus analyses of all properly treated fractions were carried out as described by Guest and Rapoport for acid soluble phosphates of blood. As a check, the total acid soluble organic phosphate was determined and compared with the sum of the separately determined fractions.

Total phosphorus determinations of tissues were conducted by the wet ashing of suitable samples. Aliquots of the neutralized diluted residues were analyzed for inorganic phosphate by the method used in the case of blood filtrates.

The water content of muscle was obtained by heating 1-2 grams of crushed frozen tissue to constant weight at 105°C.

**RESULTS.** The results of blood phosphorus analyses of control and shocked rabbits are recorded in table 1. They are in agreement with the data of Cole

et al. (2) except that the changes are more marked, a result of the use of a more drastic shock-producing treatment than that previously employed.

In tables 2-A and 2-B, the concentrations of various phosphorus fractions of the tissues of normal and shocked rabbits are compared. All values are expressed as milligrams of phosphorus per gram wet tissue with no correction for possible change in water content. The most striking changes are seen to occur in gastrocnemius muscle and duodenum, the latter undergoing reduction in total phosphorus content by loss of acid insoluble organic phosphorus. Gastroc-

TABLE 1

*Phosphorus content, hematocrit and pH of the blood of control and shocked rabbits*

Concentrations of phosphorus in the plasma and blood are expressed as mgm. %; concentrations of phosphorus in the cells as mgm. P/100 ml. cells; n = number of animals studied.

DETERMINATION	n	CONTROL	n	SHOCKED	DIFFERENCE OF MEANS†
					S.E. DIFF.
Plasma Inorganic P.....	27	6.92 ± 0.23*	11	20.0 ± 1.7	8.07
Plasma Acid Sol. Org. P.....	12	1.20 ± 0.16	5	2.35 ± 0.30	3.38
Cell Inorganic P.....	6	2.35 ± 0.18	3	7.70 ± 1.26	5.15
Cell Acid Sol. Org. P.....	6	79.1 ± 0.27	3	86.9 ± 7.1	1.03
Whole Blood Acid Insol. P.....	6	20.2 ± 1.3	3	15.0 ± 2.0	2.15
Hematocrit (% cells).....	28	43.0 ± 0.51	11	41.4 ± 1.8	0.91
pH.....	11	7.34‡	11	6.83 ± 0.03	

\* Standard Error of the mean (S.E.<sub>m</sub>) computed as  $\frac{\sigma}{\sqrt{n}}$ .  $\sigma$  is the standard deviation

of n readings computed as  $\sqrt{\frac{\sum(x-m)^2}{(n-1)}}$  where  $(x-m)$  is the deviation of each measurement from the mean.

† The Standard Error of the difference in means (S.E. diff.) is computed as  $\sqrt{S.E. m_1^2 + S.E. m_2^2}$ . The ratio of the difference of two means to the S.E. diff. is used to determine whether the two means are significantly different. When the ratio is less than 2, the difference may be adjudged statistically not significant; when it is more than 2.5 statistically significant. When the ratio falls between 2 and 2.5, the significance is somewhat doubtful. Actual probabilities may be obtained by referring the ratio to tables of the areas of the t curve.

‡ Value of Cole et al. (2).

nemius muscle shows reduction in creatine phosphate, total phosphate, total acid soluble organic phosphate, and total acid insoluble organic phosphate, while inorganic phosphate is increased.

The concentrations of the phosphorus constituents of gastrocnemius muscle of a group of control and shocked rabbits are listed in table 3. It should be pointed out that the data of table 3 differ from those of table 2 in two important respects. In previously comparing the concentrations of the phosphorus fractions (table 2), the values were expressed as milligrams of phosphorus per gram wet tissue. However, these values may undergo apparent change if the water content of the tissues of the control animal differs from that of the shocked

animal. To avoid such complication, the concentrations listed in table 3 are expressed as milligrams of phosphorus per gram dry matter. The second difference between the data of tables 2 and 3 lies in the fact that in the latter series, the values were obtained on animals shocked more severely, that is by use of the technique which involves prolonged suspension at low angles.

TABLE 2-A

*Concentrations of inorganic and organic phosphorus in 4 tissues of control and shocked rabbits*  
Concentrations expressed as mgm. P/gm. wet tissue; n = 4.

	TOTAL P		TOTAL ACID SOL. ORG. P		TOTAL ACID INSOL. ORG. P	
	Control	Shocked	Control	Shocked	Control	Shocked
Gastrocnemius muscle Limits	2.46 2.36-2.51	2.34 2.25-2.45	1.55 1.39-1.67	1.24 1.19-1.29	0.63 0.59-0.72	0.53 0.39-0.68
Duodenum Limits	3.16 3.07-3.28	2.86 2.69-3.05	0.56 0.52-0.59	0.49 0.42-0.59	2.25 2.11-2.47	2.02 1.94-2.15
Liver Limits	3.41 3.00-3.81	3.38 3.01-3.83	1.15 1.04-1.32	0.94 0.79-1.17	2.00 1.78-2.33	2.09 1.84-2.53
Kidney Limits	2.67 2.04-2.98	2.65 2.15-2.87	0.64 0.47-0.73	0.65 0.59-0.71	1.75 1.39-2.00	1.69 1.16-1.97

TABLE 2-B

*Concentrations of inorganic and organic phosphorus in 4 tissues of control and shocked rabbits*

Concentrations expressed as mgm. P/gm. wet tissue; adenosine pyro. P represents readily hydrolyzable groups; residual P includes adenylic acid; n = 4.

	INORGANIC P		CREATINE P		ADENOSINE PYRO P		RESIDUAL P	
	Control	Shocked	Control	Shocked	Control	Shocked	Control	Shocked
Gastrocnemius muscle Limits	0.28 0.16-0.38	0.58 0.48-0.69	0.46 0.28-0.65	0.10 0.01-0.16	0.31 0.10-0.49	0.29 0.05-0.38	0.72 0.53-0.89	0.76 0.61-0.98
Duodenum Limits	0.36 0.27-0.40	0.35 0.28-0.43			0.04 0.04-0.08	0.04 0.04-0.04	0.55 0.53-0.59	0.49 0.42-0.64
Liver Limits	0.26 0.18-0.35	0.36 0.27-0.51			0.08 0.08-0.11	0.04 0.03-0.05	1.04 0.88-1.27	0.94 0.77-1.24
Kidney Limits	0.29 0.18-0.35	0.32 0.29-0.35			0.04 0.01-0.08	0.04 0.03-0.05	0.65 0.42-0.79	0.67 0.56-0.81

From table 3 it can be seen that in the gastrocnemius muscle of the shocked animal, significant reductions occur in the concentrations of acid soluble organic phosphate and creatine phosphate, while the values of inorganic phosphate are increased. The increase in adenosine pyrophosphate is of doubtful significance, while the remaining constituents are not significantly altered.

When the water, lactate and glycogen content of gastrocnemius muscle, and

the glycogen content of liver of control and shocked rabbits are compared (table 4), it is found that in the shocked animal muscle lactate increases. In the case of both muscle and liver, the glycogen stores are significantly reduced. The increase in water content of the muscle of the shocked animal is of doubtful significance.

TABLE 3

*Concentrations of the phosphorus constituents of the gastrocnemius muscle of control and shocked rabbits*

Concentrations are expressed as mgm. P/gm. dry weight; n = number of animals studied.

DETERMINATION	n	CONTROL	n	SHOCKED	DIFFERENCE OF MEANS† S.E. DIFF.
Inorganic P.....	12	1.50 ± 0.06*	11	2.66 ± 0.16	7.12
Creatine P.....	11	2.02 ± 0.11	11	1.00 ± 0.18	5.25
Adenosine Pyro. P.....	11	1.34 ± 0.06	10	1.56 ± 0.09	2.37
Residual P.....	11	2.90 ± 0.16	10	2.65 ± 0.12	1.25
Acid Sol. Org. P.....	12	6.19 ± 0.14	11	5.22 ± 0.17	4.53
Acid Insol. Org. P.....	12	2.84 ± 0.13	11	2.60 ± 0.17	1.14
Total P.....	12	10.53 ± 0.08	11	10.50 ± 0.12	0.2

\* and † see table 1.

TABLE 4

*Comparison of the water, lactic acid and glycogen concentrations in gastrocnemius muscle, and glycogen concentrations in liver of control and shocked rabbits*

Concentrations of water expressed as per cent of wet weight; concentrations of lactic acid and glycogen as mgm./100 gm. wet tissue; n = number of animals studied.

DETERMINATION	n	CONTROL	n	SHOCKED	DIFFERENCE OF MEANS† S.E. DIFF.
Muscle water.....	11	76.7 ± 0.18*	7	77.7 ± 0.45	2.12
Muscle lactic acid.....	11	89 ± 11	7	211 ± 24	4.90
Muscle glycogen.....	10	534 ± 40	7	285 ± 45	4.45
Liver glycogen.....	10	1228 ± 280	12	12 ± 4.6	4.57

\* and † see table 1.

DISCUSSION. In the introduction it was pointed out that the alterations in the composition of the blood of suspended rabbits were thought to be brought about by tissue hypoxia acting to depress the aerobic phase of tissue metabolism. For example, in the shocked animal, the increase in the lactate concentration of the blood might lead one to suppose that a considerable amount of carbohydrate had been metabolized anaerobically. The demonstrated reduction of glycogen in gastrocnemius muscle and liver, and the increase of lactate in the muscle of the shocked animal, support this view. Increased peripheral utilization of carbohydrate has also been found to occur in the hemorrhagically shocked rat (7, 8, 9).

In shock, the curtailment of the aerobic phase of carbohydrate breakdown would act to reduce the rate of synthesis of energy-rich phosphate bonds, since with regard to such synthesis, this phase is five times as effective as the anaerobic phase (10). The shocked animal may then be in a condition where formation of ATP and CP is insufficient to keep pace with their utilization. Under these circumstances, one might expect a reduction of the energy-rich phosphate bond store and an increase in inorganic phosphate in muscle and blood. The data of table 3 show that in the muscle of the shocked rabbit the store of energy-rich phosphate bonds is reduced but by no means exhausted. Apparently the degradation of carbohydrate is sufficient to maintain the phosphate bond store for a time, but it may be that over a longer period these mechanisms are insufficient to provide for the needs of the animal.

Since the inorganic phosphate concentration of the gastrocnemius muscle of the shocked rabbit is markedly increased, it would seem likely that muscle would act as a phosphate donator to produce the observed increase in inorganic phosphate of the plasma. From the data herein reported this would seem not to be the case since the average total phosphorus content of the muscles of control and shocked animals is not significantly different. However if one assumes that in shock, all the muscles of the body contribute equally to increase the phosphorus content of the blood, it may be shown that the expected change in total phosphorus content of muscle is within the standard error of the obtained values. Therefore the question of whether or not inorganic phosphate of muscle acts to raise the level of inorganic phosphate of blood cannot be conclusively answered from these data.

#### SUMMARY AND CONCLUSIONS

1. Gravity shock is accompanied by extensive reduction of liver and muscle glycogen along with an increase of lactate in muscle. These changes are thought to result from depression of the aerobic phase of carbohydrate metabolism.

2. In gastrocnemius muscle, during gravity shock, the concentration of creatine phosphate decreases.

3. In gravity shock, the increase of muscle inorganic phosphate results mainly from dephosphorylation of creatine phosphate.

4. The data suggest that tissue hypoxia produced by suspension of the rabbit results in loss of energy-rich phosphate bonds of muscle, and in impairment of the mechanism which generates these bonds. Under these conditions, production of energy from carbohydrate metabolism may fall to levels insufficient to sustain the animal.

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# THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON DIETARY REQUIREMENT FOR THIAMINE, PYRIDOXINE, NICOTINIC ACID, FOLIC ACID AND CHOLINE IN CHICKS

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In the course of studies on differences in chick requirement for the anti-anemic factor in heat and cold (to be reported later), we found it necessary to determine first the requirements for the other B-vitamin fractions needed to support optimal chick growth and to prevent deficiency symptoms. Little quantitative work along this line has been reported,—none on possible differences in chick dietary needs for tropical and temperate environmental conditions.

Considerable controversy has developed over our claim that a higher dietary thiamine content (in milligrams per kilogram of food) is required to support optimal rat growth in tropical warmth than is needed in temperate coolness (1). Most of the confusion has arisen over failure to differentiate between absolute thiamine requirement in gamma/rat/day and dietary requirement as expressed in milligrams per kilogram of diet consumed. In the one case vitamin intake is independent of food consumption, while in the other it is directly affected by any factor,—such as tropical heat, advancing age or ill health,—which lowers the food intake. Since vitamins are normally ingested with the food and their intake is thus affected by all factors which influence the amount of food eaten, we have consistently adhered to an analogous approach in all our nutritional work and have thus studied the effects of varying vitamin concentrations in the diets consumed. One review journal has added to the confusion in this field by its repeated failure to consider these two phases of the vitamin requirement problem in its reviews of our various articles on the subject (2).

Since the publication of our first paper on heightened thiamine requirement (*in milligrams per kilogram of diet*) for animals adapted to tropical heat (1), several articles have been written in attempts to disprove our findings. In several instances (3) these studies were carried out on men or animals subjected to only brief exposure to tropical heat (less than 2 wks.), and hence the results could not reasonably be applied to tropical populations (4). Kline, Friedman and Nelson (5) claimed to have refuted our findings when they showed the polyneuritis-curative dose to be smaller for rats kept at 90°F. than for those kept at 75°F., but their rats were given inadequate time (7–15 days) to make metabolic adjustment to the heat before being tested. In their growth and food consumption studies, they also failed to allow for the needed adaptive period before beginning the observations.

In the most recent report along this line, Edison et al. (6) allowed ample time for adaptation but failed to incorporate into their hot room diets a sufficient amount of choline, and hence their optimal growth in the heat was always below that in the temperate environment. They used only 0.5 gram of choline per kgm. of food, while we have found that 5.0 grams/kgm. is required for best results in the heat (7). In their studies on polyneuritis in acute thiamine defi-

ciency, they used rats thoroughly adapted to the different temperatures but failed to take into account marked differences in weight. The rapidity of weight loss and onset of polyneuritis reported in their paper was in inverse relationship to body weight at the time all animals were placed on 2 gamma of thiamine per rat per day. Many years ago Cowgill (8) showed that thiamine requirement bore an essentially straight line relationship to body weight, a finding which we have recently verified with the advancing size and age of a group of rats throughout their whole life span (9). It was therefore not surprising that Edison et al. obtained earlier death and more rapid weight loss in their larger cold room rats than in the smaller ones kept in the tropical heat, when all were put on the same low thiamine intake of 2 gamma per rat per day.

Confirmation of our original findings was afforded by the studies of Sarett and Perlzweig (10), for they found that rats adapted to a 91°F. environment grew poorly and had a low-tissue thiamine content while feeding *ad libitum* on a synthetic diet entirely adequate for optimal growth response in rats kept at 75°F.

In view of all this controversy, it is of interest that our chick findings here reported also indicate a definitely higher dietary thiamine requirement (in milligrams per kilogram of diet) for the support of optimal growth and the prevention of polyneuritis in tropical warmth. For the other members of the B-complex so far studied, no significant differences in optimal dietary concentration have been found for chicks kept in tropical and temperate environments.

**EXPERIMENTAL.** In all these chick studies, day-old white Leghorn cockerels were placed on the indicated diets in an air-conditioned room maintained at 90–91°F. and 60–70 per cent relative humidity. At the end of one week, the dietary groups intended for temperate coolness were removed to a room kept at 75°F. and three days later placed in a room kept at about 70°F. Original grouping of the chicks was done on a weight basis, allowing not over 0.5-gram variations in average group weights. *Ad libitum* feeding was allowed and an effort made to arrive at a fairly close approximation of food consumption for certain of the groups (as indicated in the tables). Weekly weighings of the chicks were made and a close watch kept for signs or symptoms of deficiency. The following general dietary formula was adopted after several preliminary chick series had indicated the approximate requirements in heat and cold:

	Grams per 100 grams of diet		mgm./kgm.
		B-vitamins	
Dextrine, white commercial.....	55	Thiamine: in cold.....	3
Casein, vitamin-free.....	18	in heat.....	5
Salt mixture.....	6	Riboflavin.....	4
Soy bean oil.....	5	Pyridoxine.....	4
Gelatin.....	10	Calcium pantothenate.....	15
Extracted liver residue*.....	4	Nicotinamide.....	60
			gram/kgm.
Solubilized liver extract*.....	2	Inositol.....	1
Cystine.....	0.3	P-amino-benzoic acid.....	0.3
Hepicoleum (Lilly).....	1.2	Choline chloride.....	2
			mgm./kgm.
		Vitamin K.....	1

\* Kindly supplied by Wilson Laboratories, Chicago.

This diet formula was altered for the studies on each specific vitamin only by variation in the amount of that vitamin added for the different chick groups, such variation in the vitamin under study being indicated in the table of results in each case. All diets were kept in a cold room (at about 40°F.) until placed in the feeding pans. When synthetic folic acid became available during the course of this work, it was no longer necessary to use the liver fractions of unknown constitutions. The nicotinic acid studies here reported, and a partial repetition of the thiamine series, were carried out with diets from which the liver fractions were

TABLE 1  
*Chick growth and polyneuritis at varying levels of dietary thiamine*

THIAMINE IN DIETS	AT 70°F.		AT 90-91°F.	
	Mean body weight at 4½ weeks of age	No. developing polyneuritis	Mean body weight at 4½ weeks of age	No. developing polyneuritis
<i>mgm./kgm.</i>	<i>grams</i>		<i>grams</i>	
0.5		9/10		11/11
1.0	315.83 ± 5.56*	2/27	275.45 ± 10.44	6/28
2.0	287.50 ± 10.25	0/14	328.33 ± 12.36	2/11
3.0			338.60 ± 5.59	1/27
4.0	305.00 ± 7.30	0/14		
6.0	321.43 ± 11.77	0/14	337.86 ± 10.76	0/14
9.0			337.00 ± 9.81	0/15
10.0	292.86 ± 8.78	0/15		
12.0			358.67 ± 8.22	0/14
15.0			329.61 ± 13.72	0/14
20.0			325.00 ± 11.39	0/15
30.0			335.00 ± 8.67	0/15

\* Probable error of mean.

omitted, the dextrine raised from 55 to 61 per cent, and in which the following additional vitamins were incorporated at the levels indicated:

	<i>mgm./kgm. of diet</i>
Folic acid <sup>1</sup> .....	1
Biotin.....	0.05
A-tocopherol.....	3

*Thiamine requirement.* Using the diets still containing the liver fractions, one series of chicks were tested at thiamine concentrations ranging from 0.5 mgm. to 10 mgm. per kgm. of food in the cold and 0.5 mgm. to 30 mgm. per kgm. in the heat (see table 1).

From the data presented in table 1 it is seen that polyneuritis in the cold did not persist at thiamine levels above 1 mgm./kgm., while in the heat there was still one case at 3 mgm./kgm. The only suggestion of growth retardation at low thiamine levels was encountered at 1 mgm./kgm. in the heat. Polyneuritis was diagnosed by loss of equilibrium, backward twisting of the head, loss of muscle

<sup>1</sup> Kindly supplied by Lederle Laboratories.

co-ordination, and recovery after thiamine injection. Chicks developing polyneuritis were at once removed from the series.

Since the liver fractions contained a small amount of thiamine, we repeated this series in its lower ranges with completely synthetic diets when folic acid became available. Table 2 sets forth the results obtained. Again, it was found that polyneuritis did not persist at thiamine levels above 1 mgm./kgm. in the cold, while in the heat almost half the chicks at 2 mgm./kgm. were affected. Growth of those unaffected by polyneuritis was optimal at all thiamine levels in the cold but was significantly reduced at 1 mgm./kgm. in the heat.

This second thiamine series, as here set up, is not constituted to show whether the absolute daily thiamine requirements of chicks is different after adaptation

TABLE 2

*Chick growth and polyneuritis at varying thiamine concentrations in all-synthetic diets*

	AT 70°F.				AT 90-91°F. AND 60-70% REL. HUM.			
	0.5	1.0	2.0	3.0	0.5	1.0	2.0	5.0
Thiamine in diet (mgm./kgm.)*.....								
Wt. gain, last 3½ wks. (grams)*..	187.5 ±11.0	184.0 ±4.5	185.0 ±4.1	172.5 ±8.6	91.0 ±14.4	168.3 ±12.6	172.5 ±3.9	
Final wt. at 4½ wks. of age (grams).....	247.5 ±11.0	243.5 ±5.1	253.7 ±5.5	240.0 ±10.5	157.0 ±16.7	238.3 ±14.0	245.0 ±5.5	
Food eaten, aver. G./wk./chick.....	144	133	136	126	96	98	111	
Thiamine intake, aver. gamma/day/chick.....	10	19	39	54	14	28	79	
No. developing polyneuritis...	3/12	2/11	0/11	0/9	6/12	5/12	5/12	0/10
Deaths without polyneuritis...	6/12	4/11	1/11	1/9	5/12	2/12	0/12	1/10

\* Food intake, weight gain and final weight calculations were made only on those surviving and remaining free of polyneuritis. This also holds for table 1.

to tropical heat and temperate coolness. However, there can be no question that dietary thiamine concentrations capable of giving optimal growth and protection against polyneuritis in the cold become definitely deficient in both respects in tropical warmth.

*Nicotinic acid requirement.* Using the all-synthetic diet described above, the nicotinic acid content was varied from 0 to 100 mgm. per kgm. of diet as indicated in table 3. From the data set forth in this table, it is evident that growth rate and survival rate are not significantly different in heat and cold at analogous nicotinic acid levels in the diet. There is seen definite growth retardation and reduced food consumption at 0 and 5 mgm./kgm. and a faint suggestion of deficiency at 15 mgm./kgm. Two separate groups were run at 15 mgm./kgm. in heat and cold, to be certain no difference existed at this apparent deficiency threshold, but in neither case was any significant difference found.

*Pyridoxine requirement.* With pyridoxine concentrations of 0.5 mgm./kgm. to 4.0 mgm./kgm. in the diet, there was no noticeable difference in growth in analogous hot and cold room groups. Significant growth retardation took place in both cases only at 0.5 mgm./kgm. with one chick out of 11 dying in the cold

TABLE 3  
*Chick growth at varying dietary concentrations of nicotinic acid*

	NICOTINIC ACID IN DIET (MG./KGM.)					
	0	5	15	30	60	100
At 70°F.						
Wt. gain in last 3½ wks. (grams).....	75.0 ±12.7	140.0 ±10.2	167.0 ±8.4	188.7 ±8.9	172.5 ±8.6	
Final wt. at 4½ wks. of age (grams).....	136.0 ±12.7	208.7 ±14.0	235.0 ±9.1	252.5 ±9.1	240.0 ±10.5	
Food eaten, average (g./wk./chick).....	80	87	135	112	126	
Nicotinic acid intake, aver. (gamma/day/chick).....	0	62	289	480	1080	
Deaths after first week.....	4/8	1/9	3/18	0/8	1/9	
At 90-91°F. and 60-70% relative humidity						
Wt. gain in last 3½ wks. (grams).....	87.1 ±5.4	122.5 ±5.9	161.0 ±6.3	170.0 ±13.0	172.5 ±3.9	170.0 ±5.9
Final wt. at 4½ wks. of age (grams).....	143.3 ±6.3	185.0 ±6.6	227.0 ±6.7	243.8 ±13.4	245.0 ±5.5	235.7 ±8.1
Food eaten, average (g./wk./chick).....	46	54	117	113	111	109
Nicotinic acid intake, average (gamma/day/chick).....	0	39	251	464	951	1557
Deaths after first week.....	1/8	1/9	3/18	1/9	1/10	1/10

and 3 in the heat at this pyridoxine level. Following are the final mean body weights in grams at 5 weeks of age:

PYRIDOXINE IN DIETS	(MG./KGM.)			
	0.5	1.0	2.0	4.0
At 70°F.	258.33 ±13.02	314.00 ±11.10	352.27 ±11.58	325.91 ±9.11
At 90-91°F.	236.25 ±12.87	318.64 ±7.96	330.00 ±10.12	312.27 ±8.80

*Choline requirement.* Growth retardation in the choline series (see below) occurred only in the groups receiving 0.2 gram/kgm. From 0.4 gram/kgm. upwards, growth was optimal. The smaller body size of all groups in this series may have been due to the fact that they were summer-hatched. Only a very low incidence of slipped-tendon was encountered, none at dietary choline concentrations above 0.4 gram/kgm. in the cold and 0.8 gram/kgm. in the heat—as indicated in the following data:

CHOLINE IN DIETS	(MG./KGM.)				
	0.2	0.4	0.8	1.6	2.4
At 70°F.					
Body wt. at 4½ wks. of age	210.00	252.10	263.89	257.07	260.00
	±7.03	±7.29	±6.63	±5.21	±11.88
No. with perosis	1/9	1/31	0/27	0/29	0/12
At 90-91°F.					
Body wt. at 4½ wks. of age	226.00	246.31	247.86	257.27	240.91
	±4.98	±6.87	±7.70	±7.18	±4.87
No. with perosis	1/10	2/23	1/21	0/22	0/22

*Folic acid requirement.* These same all-synthetic diets were used in testing for differences in folic acid requirement in tropical heat and temperate coolness.

TABLE 4  
*Chick growth and hemoglobin formation at different levels of dietary folic acid*

	FOLIC ACID IN DIET (MG./KGM.)					
	0.0	0.1	0.2	0.3	0.5	1.0
At 72°F.						
Weight gain (grams) in last 4 weeks.....	129.88 ±9.53	187.00 ±10.01	172.42 ±11.29	210.56 ±8.57	194.93 ±12.54	222.57 ±10.24
Hemoglobin (grams/100 cc.).....	5.41 ±0.40	6.04 ±0.41	7.30 ±0.26	7.74 ±0.16	8.69 ±0.23	8.94 ±0.24
Number surviving....	12/25	13/24	11/13	21/24	18/25	20/23
At 90-91°F. and 60-70% relative humidity						
Weight gain (in grams) in last 4 weeks.....	116.67 ±8.55	137.45 ±17.99	181.75 ±10.94	197.14 ±10.78	187.00 ±10.19	183.60 ±11.58
Hemoglobin (grams/100 cc.).....	6.40 ±0.18	6.17 ±0.33	6.60 ±0.27	7.68 ±0.20	8.22 ±0.28	8.69 ±0.29
Number surviving...	20/24	13/23	10/12	21/25	20/25	14/23

The results set forth in table 4 fail to show any clear-cut difference. The more favorable growth at 0.1 mgm./kgm. and the higher hemoglobin level at 0.2 mgm./kgm. in the cold hint at a slightly lower requirement in temperate coolness, but they are by no means convincing without more definite confirmation.

**DISCUSSION OF RESULTS.** The chick studies here reported give definite confirmation of our earlier findings on rats showing a heightened dietary thiamine requirement (*in milligrams/kilogram of diet*) in tropical heat. Rats adapted to tropical warmth required a 2-fold increase in dietary thiamine concentration to give optimal growth, and with chicks roughly the same increase is needed. As with the rats, chick food consumption is almost 30 per cent lower in the heat.

In view of our studies showing a marked rise in rat thiamine requirements (per gram of food) with the reduced food intake of advancing age (9), it should

be borne in mind that the present findings apply only to the rapid growth of chicks during the first month of life. Investigations should be made to see whether fowls display a similar tendency with regard to thiamine requirement as they grow older, but our laboratory facilities are not adequate for handling the required numbers of larger fowls.

#### SUMMARY

The polyneuritis threshold for chicks was found at 1 mgm./kgm. of diet in temperate coolness and 3 mgm./kgm. in tropical heat. Optimal growth was obtained at 1 mgm./kgm. in the cold and 2 mgm./kgm. in the heat.

No significant differences were found in chick dietary needs for folic acid, nicotinic acid, pyridoxine or choline in temperate coolness and tropical heat.

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# EFFECT OF MILD HYPERTHYROIDISM ON SEASONAL AND YEARLY EGG PRODUCTION OF FOWLS WITH ADVANCING AGE<sup>1</sup>

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The physiological effects of thyroid hormone administration have been difficult to evaluate in the past due to a general lack of knowledge of the normal thyroid hormone secretion rate in the various species studied and the relation of the dosage administered to the normal secretion rate. It is now clear that the administration of exogenous thyroid hormone in amounts less than the normal secretion rate merely causes a depression of endogenous hormone secretion of like amount (6, 11). When exogenous hormone equals endogenous hormone, the secretion of hormone by the gland ceases. Only when exogenous thyroid hormone administration exceeds the normal rate of secretion is a state of hyperthyroidism induced.

Since the hormone of the thyroid gland appears to play rather important rôles in the process of growth, reproduction, egg laying and milk production, it is of great importance to determine whether the induction of mild hyperthyroidism will increase these productive processes, and, if so, the limits of favorable physiological effect. This is especially true since the development of methods of producing synthetic thyroprotein of high thyroxine content permits the economical induction of mild hyperthyroidism in domestic as well as experimental animals (7, 8, 9).

In 1942 a study was initiated with a group of two-year-old White Leghorn chickens to determine the seasonal and long time effect upon egg production of mild hyperthyroidism induced by the feeding of thyroprotein in the battery mash. Progress reports of our observations have been made (12, 13, 14). The feeding of thyroprotein at the rate of 10 grams/100 lbs. feed has been repeatedly observed to maintain egg production at a higher level during the spring and summer months, resulting in the production of more eggs during the year than were laid by the control chickens. During the past years the mortality rate of the two groups has been about the same.

Recently, the surviving hens in this experiment completed their fifth laying year. They have thus reached an age comparable with the 5 to 8 year old fowls reported by Crew (3) to have undergone rejuvenation through the influence of thyroid medication. However, in the present experiment all of the hens fed thyroprotein have been on continuously for two to four years, so that the effect of long time feeding should begin to appear. These data are believed

<sup>1</sup> Contribution from the Missouri Agricultural Experiment Station, Journal Series no. 1029.

to contribute to the question raised by Brody (1) whether the speeding up of the productive processes of farm animals by mild hyperthyroidism would not also accelerate senescence and by overstimulating the vital organs thereby shorten the animal's life.

**EXPERIMENTAL METHODS.** The hens were housed in a standard individual laying battery. The composition of the ration for the past three years has remained unchanged. The sample of thyroprotein<sup>2</sup> used during the entire 4 year period of the experiment was the same. It contained 2.7 per cent l-thyroxine as determined by the chemical method of Reineke et al. (10). At the level of 10 grams/100 lbs. (0.022 per cent of the feed) the birds were receiving from two to four times the amount of thyroxine secreted by two-year-old White Leghorn hens (Turner, unpublished).

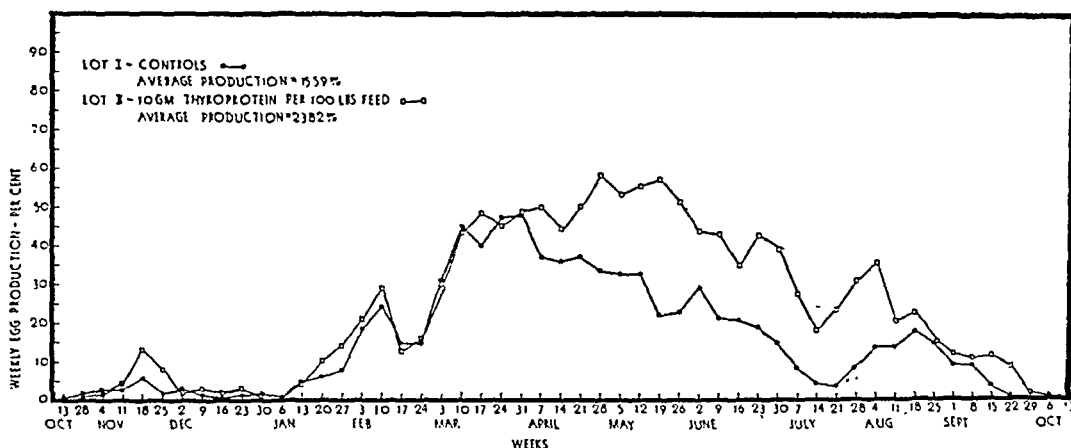


Fig. 1. A comparison of the weekly egg production of White Leghorn hens in their fifth laying year. Lot II fed 10 grams thyroprotein per 100 lbs. feed show sustained spring and summer weekly egg production in comparison with the control hens. For the entire year the thyroprotein-fed hens had a 54.5 per cent higher average weekly egg production.

The experimental years began about the middle of October. The eggs were numbered as taken from the battery, weighed and recorded. The hens were weighed each 28 days at the same time in the afternoon. For further details of the experimental procedure see the previous papers cited (12, 13, 14).

**EXPERIMENTAL RESULTS.** With advancing age, it has been observed that the control hens start egg production later and later in the fall, reach about the same peak of weekly egg production, then begin to decline in egg production earlier in the spring. The hens fed thyroprotein were quite comparable in egg production to the controls in the fall and winter but were able to maintain their egg production during the spring and summer at a considerably higher level (fig. 1).

For the entire year, the average weekly egg production of the control group was 15.59 per cent, whereas those fed thyroprotein averaged 23.82 per cent. On this basis of comparison, the thyroprotein-fed chickens had a 52.5 per cent

<sup>2</sup> The thyroprotein used in this experiment was kindly supplied by Dr. W. R. Graham, Jr., Cerophyl Laboratories, Kansas City, Mo. under the trade name of protamone.

higher average weekly egg production during the year than did the controls. It is interesting to compare these figures with comparable data for the two preceding years. During the third laying year the group fed thyroprotein laid 11.2 per cent and during the fourth year 25.7 per cent more eggs than the controls. This shows that with advancing age, the hens fed thyroprotein declined less rapidly in weekly egg production than did the control hens.

Complete data on the yearly egg production of the control and thyroprotein-fed hens is presented in table 1. In examining these data it should be noted that thyroprotein was not fed to either group during their first laying year. After the second laying year, the hens fed thyroprotein and controls were evenly divided between the two groups and a considerable number of additional hens were added. Beginning the third laying year there have been no further shifts so that the hens in the thyroprotein-fed group have been fed continuously for three years (except for five replacements starred) and six hens have been fed continuously for four years.

Using the third year's egg production as a base, it will be seen that the percentage of the previous year's total egg production with advancing age is considerably higher in the thyroprotein-fed group than in the controls.

Comparing the production of the individual hens fed thyroprotein during their 4th and 5th years, it will be noted that they declined an average of only 6.4 eggs. Of the 18 hens, nine actually laid as many or more eggs during the fifth year than they did during the fourth year. The control hens, on the other hand, declined an average of 23.5 eggs each. Of the 14 hens included only two showed an increased production during the fifth year.

Seven of the thyroprotein-fed hens laid more than 100 eggs during the current year while not one control hen laid as many as 90 eggs. The average of 58.9 eggs per bird laid by the hens fed the control feed was only 57.4 per cent of the 93.8 eggs laid by the thyroprotein-fed chickens.

In previous years the average monthly body weight of the two groups has followed the same pattern throughout the year. This year the control hens average 5.2 lbs. compared to 4.8 lbs. last year and the thyroprotein-fed hens averaged 4.9 lbs. as compared to 4.7 lbs. last year. Beginning in March, the thyroprotein-fed hens declined slightly more rapidly in average body weight than did the controls. It was at this time that the control hens began to decline in egg production. The increase in the average body weight of the control hens this year as compared to last year would appear to be due to fat deposition instead of egg production.

**DISCUSSION.** Much of the research work of the past concerning the effects of hyperthyroidism upon the glands and organs of the body and upon physiological processes such as growth and senescence, reproduction, lactation and egg production require re-evaluation in the light of recent advances in thyroid gland physiology. Past experiments in this field lacked a specific base in terms of the normal thyroid hormone secretion rate upon which to estimate degrees of hyperthyroidism. By means of the technique suggested by Dempsey and Astwood (4) and developed in a series of papers from this laboratory (5, 11, 6), it is now

TABLE 1

*Effect of thyroprotein on egg production*

Single comb white leghorns. Year October 17, 1945–October 16, 1946

NO. OF HEN	1ST YEAR EGG PROD.†	2ND YEAR EGG PROD.†	3RD YEAR EGG PROD.‡	4TH YEAR EGG PROD.§	5TH YEAR EGG PRODUCTION				
					Death	Eggs at death	Total eggs	Weight per doz. ave.	Body weight ave.
Lot I. Control feed									
8948	145	134	122	21	Sept. 27	60	6	26.0	5.1
1484	152	126	119	87			49	26.8	6.6
1342	194	136	107	72			(60)	26.9	6.5
1490	206	186	129*	47			61	25.9	5.0
1134	145	147	100	77			66	26.8	6.9
1102	201	158	118	66			68	25.9	5.7
1179	172	117	158	98			75	26.0	5.7
1163	188	155	116	96	June 8	54	50	25.4	5.3
1153	209	183	135	137			30.0	4.6	
1172	165	186	170*	56	April 25	31			5.4
2443	—	143 (I)	127	108	Aug. 11	72	(72)	26.3	4.3
2442	—	106 (I)	101	37	June 16	4		26.0	5.0
2453	—	175 (II)	148	143	April 22	33	38	26.1	3.8
2448	—	154 (II)	158	106			88	26.6	5.2
2459	—	182 (III)	116	63				25.3	5.2
2469	—	103 (IV)	109	69			61	25.6	4.8
2474	—	144 (IV)	176	83			78	26.6	3.8
2478	—	141 (IV)	177	100			53	22.9	4.3
2477	—	141 (IV)	202	100	April 18	13		26.6	3.8
Ave. of 177.7 survivors.....		148.3	136.2	82.4			58.9	26.2	5.2
% of previous prod. ....		83.5	91.8	60.5			71.5		
Lot II. 10 grams thyroprotein/100 lbs. fed									
8956	112	109	87*	118	April 29	1	99	26.0	5.1
1312	218	119	84*	93			119	25.3	4.1
1477	192	132	129*	99			76	26.3	4.1
1103	151	85	130	103			118	25.5	4.9
8943	152	112	23	5					5.2
1362	193	157	73	39			39	26.3	5.3
1369	206	128	177	163			155	24.8	4.5
1302	152	135	59*	111	June 27	24		25.6	4.8
8908	145	129	182	95	June 29	7		25.6	5.7
1106	214	160	139	127	Aug. 17	86	(86)	25.8	4.7
8959	168	150	158	99			115	23.9	4.8
1101	225	144	156	117			110	26.1	5.1
1422	216	36	83*	71			76	22.7	5.1
2446	—	92 (II)	88	61			75	26.5	5.5
2435	—	87 (I)	46	65			76	26.0	5.5
2432	—	132 (I)	118	76			96	25.6	4.0

TABLE 1—*Concluded*

NO. OF HEN	1ST YEAR EGG PROD.†	2ND YEAR EGG PROD.†	3RD YEAR EGG PROD.‡	4TH YEAR EGG PROD.§	5TH YEAR EGG PRODUCTION				
					Death	Eggs at death	Total eggs	Weight per doz. ave.	Body weight ave.
Lot II. 10 grams thyroprotein/100 lbs. fed— <i>Concluded</i>									
2456	—	91 (III)	71	65	Oct. 12	102	69	26.7	5.9
2460	—	152 (III)	232	205			142	25.5	3.7
2470	—	130 (IV)	125	127			(102)	23.0	5.3
2468	—	184 (IV)	176	148			47	29.0	5.2
2465	—	155 (III)	167	116			89	25.8	4.1
Ave. of 180.3 survivors.....		124.7	119.2	100.1			93.8	25.6	4.9
% of previous prod.....		69.2	95.6	83.9			93.7		

† Trap-nest records made at Poultry Farm.

‡ Records made in battery on present experiment except replacements which are starred (\*) which were made at Poultry Farm.

Records made in battery in individual cages. Records of nos. 1106 and 2470 are included in the average yearly production.

§ Records made in battery fed as follows: (I) Control; (II) 5 grams thyroprotein/100 lbs. feed; (III) 10 grams thyroprotein/100 lbs. feed; (IV) 20 grams thyroprotein/100 lbs. feed.

possible to establish the normal d,l-thyroxine<sup>3</sup>-equivalent secretion rate of the experimental animals to be used and to then indicate precisely the degree of hyperthyroidism involved in the experiments to be undertaken. By such standards it has been observed in fowls that up to ten times the normal secretion rate may be tolerated without serious effect on the growth rate and about four times the normal rate for laying hens.

In connection with the experimental work conducted in this laboratory, it has become clear that exogenous thyroid hormone depresses the thyrotrophic hormone and thus reduces the endogenous secretion of the thyroid in proportion to the amount administered. Thus, the exogenous thyroid hormone must exceed the normal thyroid hormone secretion rate before hyperthyroidism is induced.

In the present experiment, thyroprotein was mixed with the feed to equal about four times the two-year-old hen's thyroid secretion rate. It is presumed that the normal thyroid secretion rate would decline with advancing age, but the extent and degree of this decline is not known. On the other hand, the actual consumption of thyroprotein may have declined somewhat in proportion to declining feed consumption with age.

These data on the change in yearly egg production are believed to indicate that a mild degree of hyperthyroidism in the hen of advancing age is not harmful

<sup>3</sup> If desiccated thyroid or thyroprotein is to be fed, the amounts of these substances in the feed administered with about 0.1 per cent thiouracil required to maintain normal thyroid weights is taken to indicate the oral equivalent dosage.

to the normal reproductive process, but actually decreases the rate of senescence, to the extent that yearly egg production is a measure of senescence (Brody (2), p. 701). Since the mortality rate of the two groups of hens has been essentially the same from year to year, it would appear that the greater productivity each year, up to this time, has not been attained by over-stimulating the vital organs or by shortening the hen's life span.

It suggests, further, that the normal seasonal decline in thyroid hormone secretion rate associated with increasing summer temperature which is believed to be responsible, in part, for the seasonal decline in egg production can be prevented to a considerable extent by the maintenance of a uniform mild hyperthyroidism during this period. No evidence of greater difficulty of heat dissipation was observed in the hens fed thyroprotein during the periods of highest environmental temperature. It would seem reasonable to expect that if heat dissipation became a physiologic problem it would be reflected in reduced egg production.

#### CONCLUSIONS

A group of White Leghorn hens in their fifth laying year, fed 10 grams of thyroprotein per 100 lbs. complete feed, were observed to lay an average of 93.8 eggs each during the year. Similar control hens laid an average of 58.9 eggs each.

The hens fed thyroprotein were able to maintain egg production at a higher level during the spring and summer than the control hens. This suggests that the seasonal decline in egg production is due to reduced thyroid secretion with higher environmental temperature.

During a three year period in which thyroprotein has been fed continuously, those birds stimulated to a mild hyperthyroid state produced 11.2 per cent more eggs during the third year, 25.7 per cent more during the fourth year and 52.5 per cent more eggs during the fifth year.

It is concluded that a mild degree of hyperthyroidism continuously induced in fowls with advancing age tends to inhibit the rate of senescence (as measured by egg production) without affecting the mortality rate.

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# FACTORS CONCERNED IN THE CONTROL OF CAPILLARY PRESSURE AS INDICATED IN A CIRCULATION SCHEMA

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The schema here described was devised primarily for teaching purposes. However, it demonstrates many physical factors which apply to the circulation and yet are apt to be forgotten. In particular it shows the importance of variations in venous resistance in regulating capillary pressure. Though the circulation in an animal must be much more complex than that of the schema, the physical factors demonstrable in the latter should play a physiological rôle.

**THE SCHEMA.** The schema is a modification of one recently described by Bayliss (1940). It owes much to a method of measuring stroke volume which was devised by Krogh in 1912. In some aspects it follows principles used in an earlier schema (Bazett, 1924). It is shown semi-diagrammatically in figure 1. The *pump* consists of a simple rubber balloon housed in a glass vessel. Water entering the vessel compresses the balloon driving fluid into the valved circuit. The entrance of the driving water is controlled by a solenoid valve, which opens to connect the glass vessel with a reservoir slung at a height. This solenoid valve is then closed and another is opened. The latter allows water to escape from the glass vessel, and the balloon to refill from the schema. Thus the stroke volume of the driving system equals that of the balloon except for minor errors introduced by any distensibility in the driving system. An electric clock rotates cams which operate the solenoid valves, so that uniform pulse rates are obtained. These rates, as well as the ratio of systole to diastole, vary with the cams used. Thus in the records shown in figure 2 and table 1, experiment 1, cams 1, 2, and 3 all give a pulse rate of 20 with ratios of systole to the cycle of 1 to 4, 1 to 3, and 1 to 2 respectively, while cam 4 gives a ratio of 1 to 3 at a pulse rate of 40, and cam 5 one of 1 to 2 at a pulse rate of 80. Thus the effects of changes in ratio of systole to the cycle and of frequency may be demonstrated separately. The ratios used are approximately those obtaining in man at pulse rates of 30, 60 and 120. The actual frequencies employed are lower, since they can more readily be recorded adequately.

The curves shown in figure 2, experiment 1, indicate how closely a normal pulse curve is approached. The corresponding pressures are given in table 1. To obtain relatively normal curves of this type it is advisable to insert an air trap in the inlet system of the pump as indicated in figure 1. This not only traps air bubbles but, acting as an air cushion, damps confusing vibrations which may originate in the driving column. The *arterial system* is made up of metal, glass and rubber parts. The pump delivers to a tube with valves at either end. These

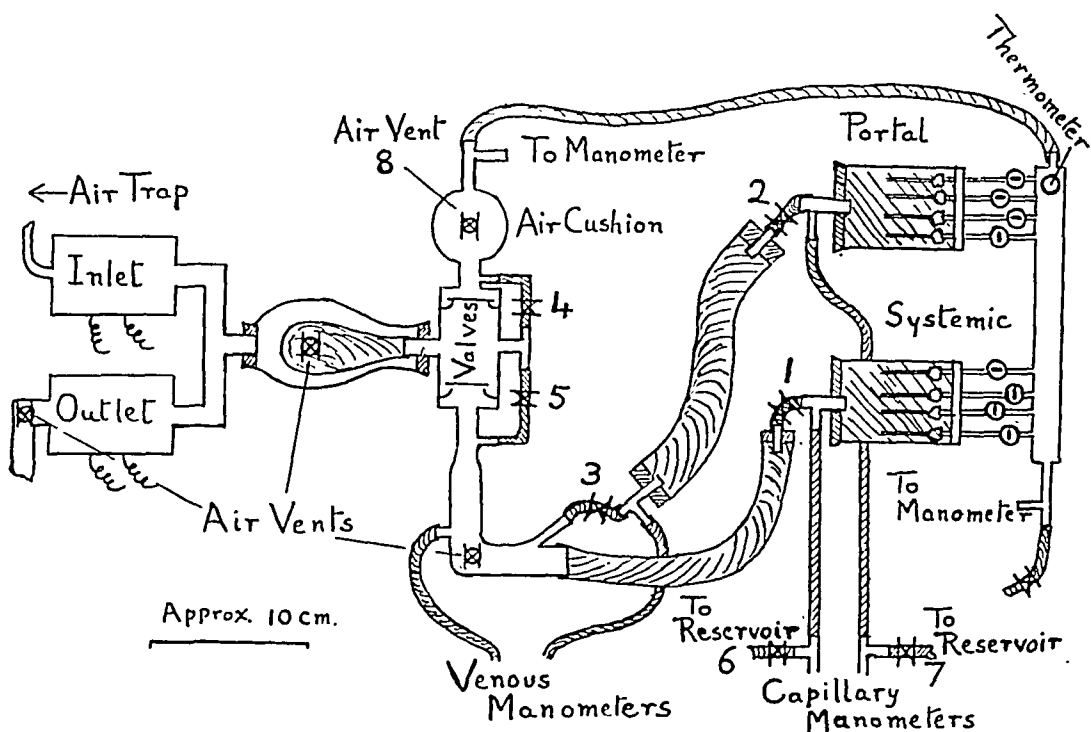


Fig. 1. A semi-diagrammatic representation of the schema is shown drawn approximately to scale. Rubber parts are shaded. Clamps 1 and 2 represent venule resistances; clamp 3 represents the resistance of the liver to portal flow; clamps 4 and 5 close tubes capable of side-tracking the two valves; clamps 6 and 7 close connections to the tissue fluid reservoirs; clamp 8 controls the vent to the air cushion.

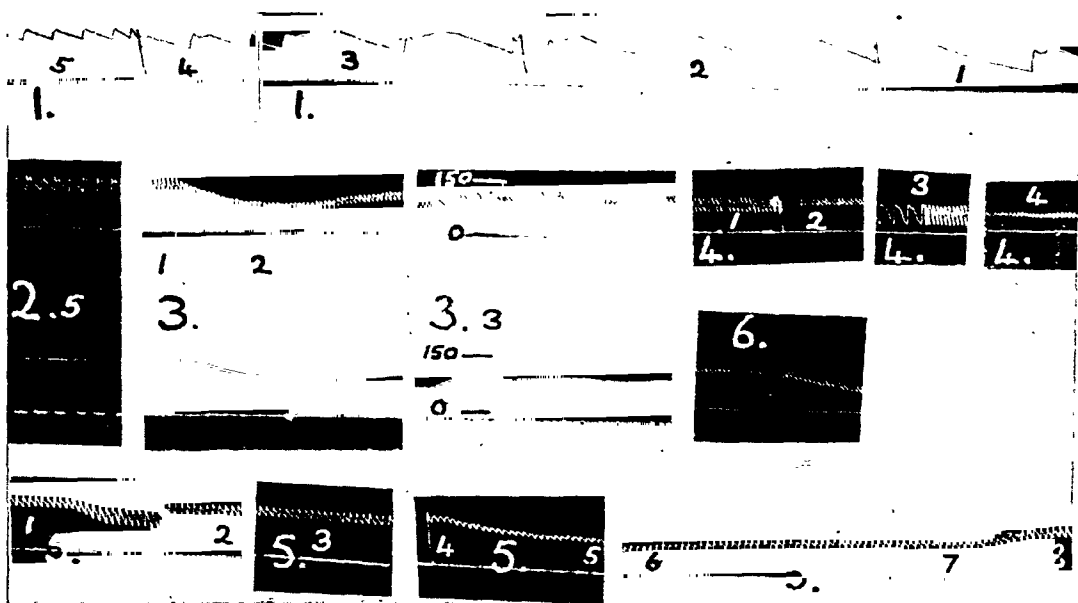


Fig. 2. Records are shown of the arterial pressures recorded in experiment 1 to 5 of table 1. The bracketed numbers of the table correspond to the smaller figures of the records marking the points at which the records were read. In experiments 2 and 3 the records from both manometers are shown. In the other experiments only records obtained by the centrally connected manometer are reproduced. Experiment 6 represents acute failure of the pump action induced by emptying of the reservoir.

valves must have low resistance and be free from leaks. They consist of brass knife edge rings, on which are seated light brass plates. The valves are arranged vertically so that the plates are seated by gravity; they are shown in

TABLE 1  
*Pressures observed in schema*

RECORD NO.	BASIC PRESS.	ARTERIOLAR RESISTANCE			PULSE RATE	STROKE VOL.	MIN-UTE VOL.	PRESSURES (MM. Hg)					
		Portal	Sys-temic	Effect. visc.				Arterial		Capillary		Venous	
								Pulse	Mean	Port.	Sys-temic	Port.	Sys-temic
1 (1)	14.8	105	35	1.11	20	8.4	168	135/65	94	14.8	14.8	14.6	13.6
(2)	14.8	105	35	1.11	20	9.5	190	148/78	108	14.8	14.8	14.3	13.2
(3)	14.8	105	35	1.11	20	9.6	192	148/91	(112)	14.8	14.8	14.0	13.2
(4)	14.8	105	35	1.11	40	5.0	200	140/95	109	14.3	14.8	14.0	12.8
(5)	14.8	105	35	1.11	80	3.0	240	153/122	130	13.6	14.8	13.6	12.0
2 (1)	13.6	35	105	0.96	40	5.0	200	125/89	101	16.2	12.3	10.5	8.7
(2)	13.6	35	105	0.96	40	5.0	200	125/87	101	14.3	12.7	9.5	7.9
(3)	13.6	105	35	0.96	40	5.1	204	129/92	106	8.9	23.2	8.6	7.8
(4)	13.6	105	35	0.96	40	4.9	196	127/89	102	10.0	17.3	7.9	7.0
(5)	13.6	105	35	0.96	40	4.7	188	122/84	98	7.0	20.0	6.8	6.3
3 (1)	15.0	105	26.3	1.04	80	3.25	260	137/101	111	12.9	17.0	-1.9	-4.4
(2)	15.0	105	26.3	1.04	80	—	—	74/56	68	12.1	17.3	-1.1	-1.8
(3)	15.0	105	35	1.04	80	Variable		As recorded					
4 (1)	14.0	105	26.3	1.0	80	2.3	184	97/55	76	13.8	14.3	11.0	9.7
(2)	14.0	105	26.3	1.0	80	2.4	192	90/70	83	13.3	14.3	10.3	8.8
(3)	14.0	105	26.3	1.0	80	1.4	(112)	78/25	53	13.1	14.3	11.7	10.7
(4)	14.0	105	26.3	1.0	80	1.0	(80)	51/38	43	13.3	14.3	11.2	9.9
						(+3.4)							
5 (1)	14.8	105	35	1.0	80	3.3	264	144/116	126	12.9	14.8	11.0	10.5
(2)	<14.8	105	35	1.0	80	3.3	264	142/111	122	10.5	12.5	1.6	1.4
(3)	?	105	35	1.0	80	3.1	248	133/107	117	1.5	3.0	-0.7	-0.8
(4)	2.0	105	35	1.0	80	—	—	130/115	120	—	—	—	—
(5)	2.0	105	35	1.0	80	2.6	208	96/83	90	12.5	15.0	—	-1.5
(6)	>2.0	105	35	1.0	80	—	—	90/75	81				
(7)		105	35	1.0	80	—	—	105/89	94				
(8)	14.5	105	35	1.0	80	—	—	144/118	126	Approximately as in 5 (1)			

figure 1 as though they were horizontal. Apart from the valve chamber, the whole schema is horizontal. Distensibility is contributed to the arterial system by a variable air cushion of about 150 m.l. maximal capacity. A single rubber tube represents the arteries. Owing to the difference in the distribution of size and distensibility, etc., in the schema from that in an animal, physiological contrasts between centrally and distally recorded arterial pulses are not pre-

served. However, the arterial side could readily be modified so as to present such features if desired (Bazett, 1924).

The *arteriolar resistances* are designed to give a quantitatively variable resistance. They are formed by eight outlets from the "artery" controlled by taps, each ending in a hypodermic needle  $1\frac{1}{4}$  inches long (25 mm.) of 19 gauge. Such needles were selected because they allow a flow of approximately 1 ml./second of water at 20°C. at a pressure differential of 100 mm. Hg. If a single unit of resistance be one which allows a flow of 1 ml. per second at a pressure differential of 1 mm. Hg, then each needle may be said to have a resistance of 100 units. The resistance to flow is determined by two factors: (a) the dimensions of the tubes, and (b) the viscosity or similar properties of the fluid. The viscosity of water varies by about 2.2 per cent per 1°C. change of temperature at temperatures of about 20°C., so that even in the schema viscosity is an important variable. Obviously the complexities introduced by the variable apparent viscosity of the blood in the circulation are absent. In the circulation additional factors are present over and above those demonstrable in the schema.

The arteriolar resistances, as estimated for a viscosity of 1 centipoise, employed in experiments illustrated in figure 2 are given in table 1, as are the pressures observed, and the estimated viscosity in centipoises as determined by the temperature. Two *capillary beds* are utilized, each supplied by 4 needles and consisting simply of broad distensible tubes of light rubber.

The *venous system* consists of two "veins", one draining the "portal" and the other the "systemic" capillary bed. Venous resistances in the form of screw clamps are provided at the central ends of each vein (fig. 1, clamps 1 and 2). These represent resistances offered by venules. On the portal vein there is a second clamp (clamp 3) placed distally to represent the resistance offered by the liver.

*Pressure measurements* are made by two membrane manometers which record blood pressure in the arterial system; one connected centrally is utilized to record pressure waves and the other connected distally to record mean pressure. The records of both manometers are shown in figure 2 for experiments 2 and 3, but only one is reproduced in the other experiments. Four water manometers are also employed to measure capillary and venous pressures as indicated in figure 1. Attempts are also made to indicate the effects of *fluid balance*. In an animal capillary pressure cannot change without inducing alterations in fluid balance according to the interaction of hydrostatic and osmotic forces. To represent this balance the water manometers recording pressure in the portal and systemic capillaries are connected with reservoirs of fluid. The pressures in the capillary and the reservoir can equalize, so that any reduction in capillary pressure allows fluid to leave the reservoir and vice versa, until a balance is again attained. The connections of the capillary with the reservoir are closed whenever a simpler system is desirable. The reservoirs consist of flasks some 6 cm. in diameter and 23 cm. in length. Thus a change of pressure within them of 1 cm. implies an alteration in the contained volume of some 25 to 30 ml. or some 5 per cent of the "vascular" contents.

**THEORETICAL CONSIDERATIONS.** The following changes may be demonstrated in this schema.

*Resting conditions.* When the pump is not functioning the whole system comes to rest at a single pressure which is in equilibrium with the pressure in the fluid reservoirs. This basic pressure is that which would be developed by a balance of osmotic and hydrostatic pressures in a circulatory system at rest, if the capillary walls remained normal. The resting pressure in the schema should be therefore some 15 to 20 mm. Hg, if it is to represent the filling of a relaxed but intact vascular bed.

*The effect of the pump action.* When the pump is started it takes fluid from the venous side and forces it into the arterial system. It thus lowers the pressure in the veins below that existing in the capillaries and raises that in the arterial system. The transfer of fluid occurs gradually and several beats must occur before the pressures reach a steady state. The rate of flow may be varied by altering the force of the beat (by raising or lowering the driving reservoir), by altering the pulse rate, by altering the ratios of systole and diastole (by changing the driving cams), or by altering the flow resistance.

*Quantitative effects of flow resistance.* The arteriolar and venous resistances in each subcircuit are in series. They may be added together to give the total resistance of the subcircuit. The two subcircuits are in parallel. These resistances ( $R_p$  for the portal and  $R_s$  for the systemic) together make the total resistance of the whole circuit ( $R$ ). This may be calculated as would be the resistance of parallel electrical circuits.

$$\frac{1}{R} = \frac{1}{R_p} + \frac{1}{R_s}$$

The pump sets up a pressure gradient, which is steeper the faster the flow, and the greater the resistance offered to this flow; it may be considered as the sum of the arterial and venous gradients. Let  $P_a$ ,  $P_{cp}$ ,  $P_{cs}$ ,  $P_{vp}$  and  $P_{vs}$  represent the mean pressures in the artery, capillaries, portal vein and the systemic vein. Let  $R_a$  be the arteriolar,  $R_v$  the total venous resistance in a subcircuit, and let  $V_t$  be the rate of flow per second. Then in either subcircuit

$$V_t = \frac{P_a - P_c}{R_a} = \frac{P_c - P_v}{R_v} \quad \text{and} \quad \frac{R_a}{R_v} = \frac{P_a - P_c}{P_c - P_v}$$

These relationships imply that if the arteriolar resistance is known and the venous is adjustable, the value of the latter may be calculated from the relation of the venous pressure drop to that on the arteriolar side. They also imply that if capillary pressure is to be maintained at a constant level during flow the ratio of arteriolar to venous resistance must be regulated. Such relationships have not received adequate attention in physiological literature.

**EXPERIMENTAL DATA.** The *basic pressure* of the whole system at rest is set by the height of the fluid in the tissue fluid reservoirs. The level used in experiments quoted is indicated in table 1.

*Pump action lowering venous pressure.* The fact that the pump raises arterial

pressure by emptying the veins is readily demonstrated. If the tissue fluid reservoirs be separated from the system (closure of clamps 6 and 7 of fig. 1) and all the arteriolar taps be closed, the pump raises the pressure on the arterial side until equilibrium is attained. There is then no flow. At the same time the pressures in the veins and capillaries are all reduced. The degree to which venous and capillary pressures are lowered is dependent on the relative capacities and distensibilities of the arterial and capillary venous sections of the system. If the distensibility of the arterial side is lowered (by decreasing the air cushion) the change in venous pressure is less for the same change in arterial pressure. If the capacity of the venous system is reduced (by shortening the tubes representing the veins), then the reduction of venous pressure is increased. When the pump is stopped the pressure changes are maintained. If the experiment is repeated after release of clamps 6 and 7 so that tissue fluid reservoirs are connected with the schema, the filling of the arterial system proceeds as before at the expense of venous contents. Yet no significant change occurs in venous pressure since any fluid lost from the veins is replaced by "tissue fluid". Stasis occurs with a full dilatation of the heart and veins. The condition then generated resembles the state of an animal, if heart action is suddenly prevented by clamping the aorta, though in this case the coronaries provide a leak.

The effect of an active circulation in lowering venous pressure may also be readily seen. In experiment 1 (though designed primarily to show wave form) a moderate variation in "cardiac" output occurred. The pressures observed are shown in table 1. With greater output not only was arterial pressure raised to a greater height, but also lowering of venous pressure was exaggerated. If the pump fails, as it may do spontaneously if the driving reservoir runs out of fluid, blood pressure rapidly falls as may be seen in experiment 6 of figure 2. The fall in arterial pressure is accompanied by an equally precipitous rise in venous pressure.

*The effects of venous resistance on capillary pressure* may be readily demonstrated by altering the distribution of arteriolar resistance without readjusting venous resistance. Thus in experiment 2 of table 1, the initial setting for (1) was 3 needles open in the portal circuit, and one in the systemic. The venous resistances were adjusted experimentally to give the pressures indicated in the table. The connections to the tissue fluid reservoirs were closed. In record 2 these connections to the reservoirs were reopened. Pressure in the portal capillary was not as high, and in the systemic not as low, as fluid exchange occurred between the capillaries and reservoirs. Pressures in both systemic and portal veins were lowered, since more fluid was being discharged into the portal reservoir than was being absorbed in the systemic, decreasing the distention of the whole vascular bed.

In record 3 the arteriolar resistances were reversed without making any other change except that the connections to the tissue fluid reservoirs were again closed. Three systemic paths were then open and only one portal. Such a reversal, unaccompanied by alteration of the venous resistances, effected a slight increase in total resistance, as may be readily calculated. At the same

time capillary pressure was greatly raised in the systemic and lowered in the portal circuit. In record 4 the tissue fluid reservoir connections were again released. This minimized the changes in capillary pressure. Fluid was rapidly transported from the portal to the systemic reservoir until one was empty and the other full. Thus a new condition was set up which approached a steady state at the time record 5 was obtained. The actual fluid transferred to the systemic was greater than that taken from the portal reservoir (indicated by a rise in pressure in the systemic reservoir of 7.3 mm. of Hg and a fall in the portal of only 6.7 mm. of Hg, implying a loss of fluid from the vascular system to the reservoirs of some 25 ml.). This fluid transfer accounted for the fall in venous pressure demonstrated at stage 5. The arterial pressure recorded at this stage is shown in figure 2, experiment 2 (5). Records obtained in the earlier stages were throughout indistinguishable from this record. In this experiment capillary pressures were later readily returned to basic levels by lowering the venous resistance in the systemic and raising that in the portal system.

It is possible to calculate and predict the effects produced, and the observed data agree with such calculations. However, venous resistances imposed by clamps generate turbulent flow, so that such resistances are somewhat increased when the flow is faster.

*Quantitative estimates of resistance and their effects on capillary pressure.* The general relations are best indicated by utilizing the experiment just quoted as an example. The resistance values of each needle together with its connecting tube and tap was 105 units. Consequently in stage 1 the estimated resistances (after allowing for the temperature) were 33.7 for the portal and 101 units for the systemic subcircuit. The inflow into each capillary bed should have been  $\frac{101-16.2}{33.7}$  and  $\frac{101-12.3}{101}$  respectively. These give theoretical flows of 2.51 and 0.88 ml/sec, or a total flow per minute of 203 as compared with an observed flow of 200 ml. There was a fall of pressure between portal capillaries and portal vein of 5.7 mm, between portal and systemic vein of 1.8 mm, and between systemic capillaries and vein of 3.7 mm. The value of the respective venous resistances calculated from the estimated rate of flow and these pressures were 2.3, 0.7 and 4.1 respectively. The total venous resistance was in the portal 3.0 units, and in the systemic 4.1 units.

In stage 2 (see table 1) conditions were little changed, except that venous pressures were lowered by loss of vascular contents into tissue fluid reservoirs, and equilibrium had not been fully attained.

In stage 3 with reversal of arteriolar resistances and no change in venous resistance the value of the total resistance was changed from 27.1 units to 27.7 units. Since the pressure gradient from artery to systemic vein was 98.2 mm., the flow should have been  $\frac{98.2}{104}$  for the

portal and  $\frac{98.2}{37.8}$  for the systemic subcircuit or 0.94 and 2.6 ml/second respectively, giving a total flow of 212 ml. per minute (observed 206). (The slight increase over previous values at a higher resistance depended on a refilling of the driving reservoir.) The pressure drop between the systemic capillaries and vein is then calculable as  $2.6 \times 4.1$  mm., so that the systemic capillary pressure should have been  $7.8 + 10.7$  or 18.5 mm. The observed value was much greater, as might be anticipated, since the venous resistance would have been exaggerated by turbulence with the faster flow. On similar reasoning the pressure in the portal vein is by calculation 8.5 mm. (observed 8.6) and the pressure in the portal capillaries 10.7 (observed 8.7). The resistance between the portal capillaries and portal vein should

be less than that calculated as the result of reduced turbulence. The agreement between theory and observation was therefore good. It is clear that failure to match changes in arteriolar resistance with parallel changes in venous resistance caused marked alterations in capillary pressure.

The fluid exchanges complicating the picture when clamps 6 and 7 were released have already been considered on a qualitative basis. They need not be reiterated.

*Simulation of hemorrhage.* The effect of venous resistance in modifying both capillary pressure and the circulation as a whole may be seen in experiments on "hemorrhage" with the connections to tissue fluid reservoirs closed, as shown in experiment 5 of figure 2 and table 1. The initial stage of 5 (1) was modified by "bleeding" 40 ml. The final result was a slight reduction in arterial pressure, a considerable reduction in capillary pressure and a great reduction in venous pressure. A marked emptying of the vein was very noticeable. This effect depended mainly on a lowering of the whole basal pressure. The change in venous pressure was not only proportionately, but also absolutely, greater than in other parts of the system. In the record reproduced the temporary effect of lowered peripheral resistance on arterial pressure as fluid was withdrawn is indicated between points 1 and 2, which alone are analyzed in the table. At stage 3 an additional 33 ml. was being withdrawn gradually and had proceeded about  $\frac{2}{3}$  to completion. The effect on arterial pressure was still small, though the veins had become very empty. (The exit tube from the pump throughout was set some 10 cm. below the level of the balloon, so that diastolic pressure within the "pericardium" was of the order of -7.5 mm. Hg.) It will be noted that capillary pressures were very low and venous pressures subatmospheric, yet the pump functioned well. Under normal conditions the pump action is but little affected by gravity, if the schema be tilted up or down; under conditions such as those of stage 3 it becomes extremely sensitive. Tilting the pump end up through an angle of 30° may cause the whole action to cease, while tilting in the opposite direction greatly aids it. These effects were not demonstrated in this particular experiment. The pump action was stopped. The basic pressure level regained after the second withdrawal of fluid was less than 2 mm. Hg.

The resistances in the venous channels were then increased so as to raise capillary pressure during flow. The record of stage 4 indicates the changes when the pump was restarted after this readjustment. Initially the balloon was well filled, and arterial pressure was raised to a good height in some 10 beats before the graphic record was started. Thereafter the circulation began to fail. Fluid was displaced into the capillary beds, as well as into the artery, thus depleting the veins beyond an effective level. A vicious circle was developed and at stage 5 conditions were as indicated in table 1 (though the stroke volume was being reduced and the actual volume at this moment was uncertain). At this stage the clamps preventing entrance of tissue fluid were released. In spite of this, venous pressure continued to fall and the arterial systolic pressure reached a level of 74 mm., though capillary pressure appeared to be rising. A little later there was definite but slow recovery in all pressures as may be seen at stages 6 and 7. At stage 7 the venous resistances were again lowered to about their previous level,

allowing the capillary filling to spread rapidly to other areas. The rapid recovery to stage 8 occurred. The possible physiological significance of such reactions is discussed later. The data make it abundantly clear that variations in venous resistance may have profound effects on a system such as that of the schema.

*Duplication of other physiological conditions.* Before discussing the significance of the results, attention may be drawn to a few other records indicating the teaching value of the schema.

Record 3 in stages 1 and 2 illustrate the effect of raising pressure in the "pericardium" by manipulating the level of the exit tube of the driving system. At stage 1 this level was 10 cm. below that of the balloon inducing a diastolic negative pressure within the pericardium of some -7.5 mm. Hg. The venous resistances utilized were relatively high, so that the central veins were at low pressure and relatively empty. Raising pericardial pressure to the atmospheric level induced marked reduction of the output as indicated by the arterial pressure record shown in figure 2, experiment 3 (2). The record of experiment 3 (3) was obtained on another occasion under somewhat similar conditions. It shows how changes in arterial pressure produced by variations in intrapericardial pressure may simulate some types of respiratory variations of arterial pressure. It should be emphasized that such changes are not seen with moderate changes in intrapericardial pressure, when the veins are well filled and venous return is fully adequate. Only when the veins are badly filled are such effects readily produced.

The records of experiment 4, stages 1 and 2, indicate the effect of *arterial distensibility* on the system. The records of stage 1 were obtained with an abnormally indistensible arterial system. For stage 2 the distensibility had been returned to its normal level. As arterial distensibility was increased the lowering of venous pressure by the pump was exaggerated.

The record of experiment 4, stage 3, was obtained by merely releasing clamp 4 completely, thus allowing a marked "aortic regurgitation". The apparent stroke volume was 5.4 ml. The volume actually circulating may be estimated approximately from the known resistance and the pressure gradient; the difference represents pendulum fluid and it is given in brackets in table 1. A few pulses were recorded on a moderately fast drum to demonstrate that the distortion of the pressure changes was a diastolic phenomenon. The record of stage 4 of this same experiment shows the effect of a similar complete release of clamp 5 to give "mitral regurgitation". The other clamp had been closed. The values observed are given in table 1.

An additional factor may be demonstrated though it is not illustrated in the figures. If the schema be warmed the viscosity of water is lowered, and the whole resistance is reduced, while the needles remain unaltered. The effect is mainly seen in increased pulse pressure and flow.

**DISCUSSION.** The importance of venous resistance in this regulation of capillary pressure has received little attention in the literature except from Krogh (1929). He conceived of an automatic regulation through increase in venous resistance when flow is reduced and the veins partially collapse. While this factor is undoubtedly important, it is difficult to believe it the sole mode of control. It is not reproduced in the schema. The schema also suffers from a failure to behave according to Starling's law. The whole circulation is modified by the basic pressure to which it is filled, as was the schema described by Starr and Rawson (1936), but this does not reproduce the effect of filling on pump action.

The effects of venous resistance might be suspected of being exaggerated in the

schema; on the contrary they are probably minimized. Accepting as a standard Landis' (1930) figures for resting pressure gradients in the rat, the normal pressure gradient from large arteries to the venous end of a capillary is some 82 mm. The total resistance along the whole venous channel on such data would represent some 15 per cent of the total resistance of the circulation. In experiment 2 (1) the maximal venous resistance was 8.2 per cent of the total. In experiment 3 (1) it was higher, reaching, in the systemic subcircuit, 18.5 per cent of the total.

Another effect of venous resistance is worth consideration. The initial effects of hemorrhage in the schema when capillary and venous pressures were allowed to fall as in experiment 5, stages 2 and 3, were less serious as far as "cardiac output" was concerned, than were those after venous resistance had been increased. If initially the arteriolar resistance had been lowered and arterial pressure had been allowed to fall, the fluid saved from the arterial and capillary systems, and so made available to the veins, would have been even greater. Increasing the distention of the capillaries by increased venous resistance made the emptying of the veins critical. The sequence of events in this schema experiment cannot but be regarded as possibly illustrating in reverse the physiological changes demonstrated by Barcroft et al. (1944) in their analysis of the fainting reaction to hemorrhage. In their subjects a maintained blood pressure associated with pronounced general vasoconstriction changed to local dilatation with a marked fall in pressure. Yet cardiac output increased. It seems possible that their later condition resembled that of experiment 5 (3) with the addition of local arteriolar dilatation. Such a state, while depriving the subject of resistance to gravity effects, might considerably lighten the task of the heart.

The schema is also able to show the displacement of fluid from the portal system to the systemic veins which may be induced by simple increase in arteriolar resistance in the portal system. It was for this purpose that Krogh devised his schema in 1912.

The factors causing variations in central venous pressure in the circulation are difficult to analyze, while in the schema they may be more readily separated and identified. Failure of the pump invariably reduces the arterial-venous pressure gradient if the resistance is not changed, but the effect may be neutralized or reversed by an increase in resistance. The pressure level at which this gradient is set up is affected by the basic pressure to which the system is filled when at rest. The degree to which central venous pressure is lowered depends on the fluid displaced from it to the arteries and is less the greater the capacity and distensibility of the venous system. The level, at which capillary pressure is found on the pressure gradient between artery and vein, is determined by the ratio of the resistances central and distal to the capillary. The fluid balance at the capillary pressure level determines the intake or output of fluid and the summated effects of these exchanges in the two capillary systems determines the basic pressure level in the working state and so modifies the other pressures (as may be seen, for instance, in expt. 2 (5)).

Emphasis need not be placed here on the evidence readily obtained in the schema of conditions in which pulse pressure changes give good indications of those in cardiac output, nor of other conditions, when the shape of the pulse

curve is altered, where such agreement is absent. Suffice it to state that such a schema is able to teach a critical attitude towards mathematical deductions, provided that the additional complexities introduced by viscous or plastic properties of blood be remembered. In conclusion the use of the schema as a method of analysis may be justified on the basis of its possibilities as a promoter of quantitative thought. Such analysis in the schema is beyond criticism, but in the circulation itself it can only develop slowly. However, attempts at quantitative analysis, even if tentative and inaccurate, are worth more than hazy generalities that make no such attempt.

#### SUMMARY

1. A schema is described in which the pump output is readily measured. Comparison of this output with the overall pressure gradient allows calculation of the resistance to flow. This resistance consists of "arteriolar" and "venous" elements; the former can be regulated and set at a desired level; the latter can be estimated quantitatively from the relative pressure gradients in the two parts of the system. The schematic capillary can exchange fluid with a fluid reservoir connected to it to allow investigation of the hydrodynamic effects of such exchange of fluid across a capillary wall.

2. The schema demonstrates that changes in arteriolar resistance must be matched with equivalent changes in the venous resistance, if flow is to be directed into different channels without disturbance of the capillary pressure levels.

3. The existence of fluid exchange between fluid reservoirs and the vascular system modifies but does not nullify such conclusions.

4. A shift in the distribution of blood flow in the body is likely to be associated with minor changes in capillary pressure and alterations in tissue fluid distribution, but such changes are major, and not minor, unless venous resistance is regulated.

5. The automatic control of venous resistance as the result of variations in frictional forces in the veins according to their degree of filling, a theory advanced by Krogh, is discussed briefly. The schema is not adapted for testing this hypothesis.

6. Other physiological factors which may be demonstrated by the schema for teaching purposes are mentioned.

7. A plea is made for more quantitative analysis of the circulation, even when conditions make deductions relatively inaccurate, provided that conclusions be not drawn beyond the accuracy attainable.

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# EXCESS VITAMIN A INGESTION, THYROID SIZE AND ENERGY METABOLISM

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A curious effect of heavy vitamin A administration is that it depresses basal metabolism (1). The mechanism thereof is not known but it seems to be associated with the endocrine system. This paper presents quantitative data on the effect of heavy vitamin A administration, alone and in combination with thyroxine or thiouracil, on some metabolic and anatomic responses of white rats, together with attempts to interpret the interrelations.

**METHODS.** Metabolic and anatomic observations were made on the following groups of white rats: controls; fed percomorph oil containing about 30,000 I.U. vitamin A per day; injected 1 mgm. thyroxine per kgm. body weight at the beginning of each weekly experiment; injected thyroxine and fed percomorph oil in the above dosages; fed thiouracil (feed contained 0.1 per cent thiouracil); thiouracil plus vitamin A; dinitrophenol 2.5 mgm. per kgm. body weight; dinitrophenol plus vitamin A; partly and completely oxidized vitamin A.

**RESULTS.** The effect of vitamin A, of thyroxine, and of combinations of the two on basal metabolism are graphed in figure 1 and the effects on thyroid size are listed in table 1.

Figure 1 shows that vitamin A in large doses depresses the rate of oxygen consumption of normally-fed rats by about 10 per cent; and it depresses the metabolism of the thyroxine-injected rats by about 20 per cent. These results have been confirmed by repeating the experiments several times.

Feeding potassium iodide lowered slightly the metabolism in the normal but not in the thyroxine-treated rats.

Table 1 shows that the rats that received the excess vitamin A supplement had smaller thyroids than those that did not get the vitamin A supplement. The males were affected somewhat less by the vitamin A than the females or the castrated males. The sex difference, however, was not great.

"Completely oxidized" vitamin A (air bubbled through the hot oil for 6 hrs.) had no effect; but "partly oxidized" vitamin A (air bubbled 1 to 2 hrs.) exerted the thyroid-reducing effect.

**DISCUSSION.** Table 1 and figure 1 demonstrate in a quantitative manner that excess vitamin A depresses the metabolic rate and reduces the thyroid size of normal, thiouracil-treated and thyroxine-treated rats. What are the mechanisms of these effects?

Belasco and Murlin (2) suggested that since vitamin A has a double bond, it may become iodinated and relieve certain hyperthyroid conditions, such as increased metabolic rate, as do some other iodine compounds. This suggestion

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was tested by feeding potassium iodide to the thyroxine-treated rats. The potassium iodide depressed slightly the metabolic rate of the controls but not of those treated with thyroxine.

We should like to suggest that when vitamin A is fed in excess, its double bond takes up the iodine from the thyroxine, thus rendering the thyroxine ineffective and thereby reducing the metabolic rate. The iodinated vitamin A so formed may, however, depress the secretion of the anterior pituitary thyrotrophic hormone as thyroxine does, and thus diminish thyroid size. This seems to explain quite simply how excess vitamin A reduces thyroid size (table 1) by depressing

TABLE 1

*The influence of vitamin A, thyroxine, thiouracil, and dinitrophenol on thyroid weight*

SUBJECT	NO. OF RATS	AVERAGE BODY WEIGHT	THYROID WEIGHT MGM. PER 100 GRAMS BODY WT.			DE- CREASE IN THY- ROID WEIGHT	STATIST. SIGNIF.
			Aver.	Stand. dev.	Range		
		gram				%	
Control, female.....	10	148.5	10.05	1.329	7.34-11.98		
Vitamin A, female.....	10	147.3	6.57	1.030	4.87- 8.61	35	38*
Thiouracil, female.....	10	138.7	34.23	4.654	28.27-40.49		
Thiouracil plus vitamin A, female.....	10	170.4	27.48	4.125	23.11-37.19	20	11*
Control, male.....	8	208.5	8.18	0.248	7.21- 9.31		
Vitamin A, male.....	8	228.0	5.72	0.102	5.33- 6.27	30	61*
Dinitrophenol, male.....	8	179.1	5.22	0.171	4.45- 6.20		
Dinitrophenol plus vitamin A, male.....	6	183.3	4.81	0.122	4.38- 5.62	7	2(N.S.)
Control, castrated.....	10	158.4	7.42	0.079	6.96- 7.80		
Vitamin A, castrated.....	7	206.6	4.86	0.117	4.43- 5.50	36	256*

\* There is less than one chance in one hundred trials that the differences between thyroid weights could have arisen by chance. The tests for significance were made by means of Snedecor's Tables (see Snedecor, G. W., *Statistical methods*, p. 174, 1937).

N.S. = not significant.

the production of thyrotrophic hormone; and how it depresses the metabolic rate (fig. 1) by inactivating the thyroxine.

Other simple explanations may be suggested which, however, may be criticised. For instance, the recent dramatic developments in the field of anti-vitamins, as illustrated by the effectiveness of the sulfonamides and related substances in displacing essential vitamins, suggests that excess vitamin A may act analogously as an anti-thyroid. Vitamin A in excess may displace thyroxine from metabolically essential systems and thus reduce the metabolic rate. The thyroxine thus set free will depress the production of thyrotrophic hormone and therefore reduce thyroid size. The objection to this theory is that excess vitamin

A also decreases the thyroid size of thiouracil-fed rats (table 1), that is, when the thyroxine stores were presumably exhausted. This objection does not hold in the preceding theory in which it is assumed that the iodinated vitamin A, not the thyroxine, depresses the secretion of thyrotrophic hormone and therefore reduces the thyroid size. We are attempting to determine the effects of iodinated vitamin A on thyrotrophic hormone secretion, that is, on thyroid size.

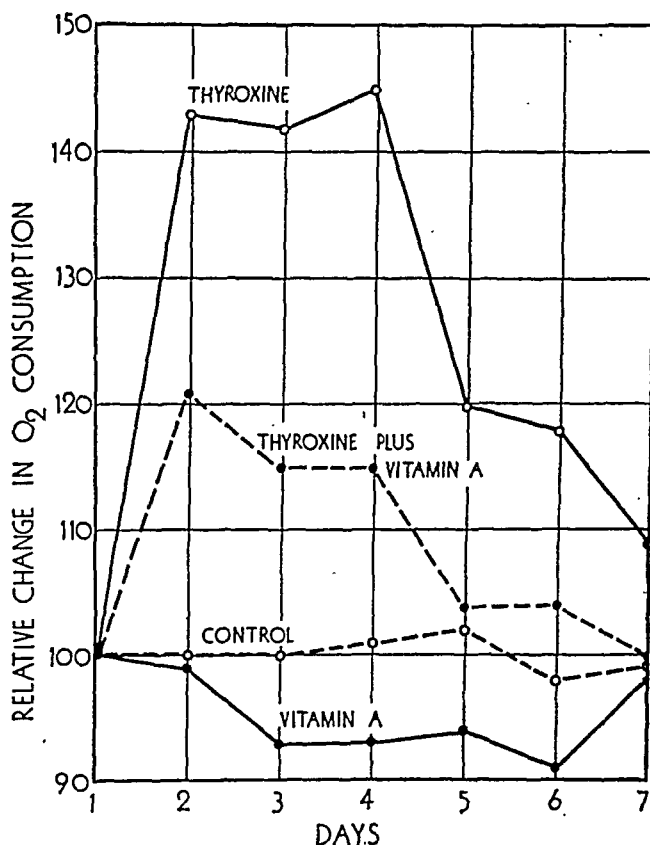


Fig. 1. Influence of vitamin A, thyroxine and thyroxine plus vitamin A administration on oxygen consumption. The thyroxine was injected once, at the beginning of the experiment, at the rate of 1 mgm. per kgm. body weight. The vitamin A was fed daily, in the form of percomorph oil, at the rate of 30,000 I.U. per rat per day.

Other, more far-fetched, explanations come to mind. For instance, since vitamin A has an unsaturated linkage, it may act as a redox perhaps as a part of an electron-transfer system and compete with some oxidative enzyme. The vitamin A may thus depress tissue oxidation indirectly and reduce the metabolic rate, just as if the thyroid function were depressed. This hypothesis was tested, rather crudely, by partly oxidizing the vitamin A as previously explained, when it continued to depress the thyroid size; but if the vitamin A was completely oxidized, the thyroid-depressing effect was lost.

Having established the fact that vitamin A depresses the metabolism-stimulating effect of thyroxine one wonders whether vitamin A might not similarly

depress the metabolism-stimulating effect of dinitrophenol. This idea was subjected to experimental test but could not be demonstrated perhaps because, unlike thyroxine, dinitrophenol acts very rapidly. Moreover (3), dinitrophenol is toxic, it blocks normal glycogen synthesis and upsets the normal course of the metabolic sequence.

#### SUMMARY

1. Heavy vitamin A medication, while allowing normal growth of the rats, depressed their basal metabolism and reduced the weight of their thyroids. The excess vitamin A also depressed the thyroid size of thiouracil-treated rats.

2. Heavy vitamin A medication tends to neutralize the increased metabolic effect of thyroxine injection.

3. While potassium iodide decreased somewhat the metabolic rate of control rats, it did not decrease the metabolism of the thyroxine-treated rats.

4. Partly oxidized—but not fully oxidized—vitamin A reduced the thyroid size.

5. Several theories are suggested for the observed effects of excess vitamin A ingestion in depressing the metabolic rate and thyroid size; the simplest is that the thyroxine iodine is taken up by the double bond of the vitamin A. This reaction removes the metabolism-stimulating effects of thyroxine and the resulting iodinated vitamin A, like thyroxine, depresses pituitary thyrotrophic hormone secretion, thereby reducing the thyroid size.

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# SOME ENDOCRINE INFLUENCES ON RENAL FUNCTION AND CARDIAC OUTPUT

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Influences of the adrenal cortex on renal management of water and electrolytes and of the neurohypophysis on water exchange have been studied for a considerable period of years; only recently have appeared systematic studies of various endocrine influences on glomerular filtration rates<sup>2</sup>, on tubular excretion and reabsorption of certain organic solutes, on renal circulation<sup>2</sup> and on daily urine output.

We have reported (1) (8) (9) that within a few days after total or simple hypophysectomy<sup>3</sup> both Diodrast (D) and inulin plasma clearances drop to 50–60 per

<sup>1</sup> Recipients of a grant-in-aid from the Commonwealth Fund.

<sup>2</sup> In the dog, the term "glomerular filtration rate" refers to creatinine, inulin or mannitol plasma clearance. We have proposed (1) that diodrast (D) plasma clearance  $\times 1.2$  is a better measure of renal plasma flow than is D plasma clearance. This was based on the premise that

$$\frac{\text{inulin plasma clearance}}{\text{inulin plasma extraction}} \quad \text{or} \quad \frac{D \text{ whole blood clearance} \times V_p}{D \text{ whole blood extraction}}$$

is a true measure of renal plasma flow; the renal plasma flow so obtained averaged 1.19 times as great as D plasma clearance. This premise is valid provided (a) the renal vein blood collections are technically perfect and representative of the mean values throughout the clearance periods, and (b) the observed plasma hematocrit fraction,  $V_p$ , is representative of that of all the blood passing through the kidney during the clearance period. We also reported (2) that the D plasma renal extraction ratio at low plasma levels averaged 0.74; it is our present belief that our higher values, 0.85, are more nearly correct than is our average of 0.74, where values as low as 0.61 were included. This belief is based on the facts (a) that any conceivable error, either in renal vein blood collection and handling or inherent in the preparation, could work only toward creating an erroneously low observed extraction ratio, and (b) that several of our lower values were obtained at plasma I levels between 6 and 13 mgm. per 100 cc., and on subsequent findings (3) of 0.84 for D and (4) of 0.87 for para-amino hippurate (PAH) on similar preparations. This belief is further supported by the findings (5) that directly observed renal blood flow in the dog averages only 10 per cent higher than that calculated from PAH clearances and hematocrit readings and (6) that PAH plasma extraction ratios in man averaged 0.88; the identity of D and PAH plasma clearances indicates identity of their renal extraction ratios. The question of whether D and PAH plasma clearances by normal kidneys should be multiplied by 1.19–1.20 (1), by 1.15 (3) (4), or by 1.10 (5) is, of course, of minor importance as compared with that of possibly large and varying corrections for abnormal kidneys; in this paper we are therefore simply presenting the data on D plasma clearances, with the belief that they are about 90 per cent of renal plasma flows.

<sup>3</sup> Total hypophysectomy is complete removal or destruction of all divisions of the hypophysis, including median eminence and stalk; simple hypophysectomy is cutting of the stalk with removal of the dependent gland, leaving pars tuberalis, median eminence and proximal stump of stalk. Denervation of neurohypophysis is eventually functionally equivalent to removal of all the neurohypophysis, including median eminence and stalk.

cent of normal and remain there permanently; filtration fraction remains normal. The drop in D clearance is not due to a drop in renal extraction of D, which remains normal. Maximum tubular excretion of diodrast, D Tm, drops within a few days to 50 per cent of normal, with a further decline to 20–30 per cent of normal within a few weeks, where it remains permanently. There is no significant change in plasma or blood volumes when calculated on the basis of pre-operative weights, although there is a fall on the basis of weights at the time of observation. There is no increase and frequently a decrease of 10–15 mm. Hg in systolic and diastolic pressures by the auscultatory method.

With denervation of neurohypophysis<sup>3</sup> (7) (9) (puncture) there is an immediate slight (10–15 per cent) rise above normal in D and in inulin or creatinine plasma clearances, followed by a slight (20–25 per cent) fall below normal at 4 to 6 weeks, followed by a return reaching to or almost to normal by 6 months. Urea plasma clearances follow creatinine but with somewhat larger percentile changes; by 6 months urea clearance has returned to or almost to normal. Plasma urea rises to 50–100 per cent above normal during the first few weeks but has returned to or almost to normal by 6 months. Within a few days following puncture, D Tm may fall slightly (20–30 per cent) but returns to normal within a few weeks and remains there permanently. These transitory falls in renal plasma flow, in glomerular filtration rate and in D Tm, with recovery within a few weeks, are ascribed to slight operative reversible damage to the pars distalis. There is no change in plasma or blood volume.

Anterior lobe extracts (11) increase D and inulin clearances and D Tm in normal, in simple hypophysectomy and in total hypophysectomy dogs and in puncture dogs, with greater increases in the hypophysectomy dogs, where these functions were depressed, than in normals or punctures; there was no significant difference in responses of dogs with simple as compared with total hypophysectomy. Thyroid, 0.1 gram/kilo/day orally, brings on a slight rise in D and inulin clearances in normal and puncture dogs, and greater rises (up to 60 per cent increase in inulin and 100 per cent in D) in both types of hypophysectomized animals. It increases D Tm in normal and puncture dogs by 25–50 per cent, with much greater increases (up to several hundred per cent) in both types of hypophysectomized dogs. The finding of normal functions in puncture dogs, with a regression of thyroid due to loss of basophils (12), is consistent with the view that the normally exhibited influence of the thyroid on these renal functions is not great. Adrenal cortical extract produced little or no change in D and inulin clearances in all dogs except punctures, where there was a slight increase; D Tm was unchanged or slightly decreased in all types.

Phlorhizin, 0.2 gram per kilo subcutaneously, which raises glucose clearance to about 85 per cent that of inulin clearance in unanesthetized dogs, has no effect on renal plasma flow as determined by renal extractions of inulin or D, lowers D plasma clearance even at low plasma levels, lowers D Tm to about 60 per cent of normal and slightly lowers inulin clearance; the tubular transport of D at both high and low plasma levels is about 60 per cent of normal. All phlorhizin observations were on normal unanesthetized dogs (10).

There is no significant effect on creatinine or D clearances in normal female

dogs on daily administration of 100 mgm. or more of testosterone propionate; D Tm showed a large and prompt rise, the effect outlasting the period of administration by a greatly variable period. Daily outputs of both sodium and potassium were decreased; blood pressure was unchanged (13).

In man, either with normal or with impaired kidney function, testosterone or testosterone propionate did not affect mannitol or PAH clearances, or glucose or PAH Tm (14). Pregnenalone (Schering) had no effect on the above functions in man, nor did orchietomy in 4 men. Daily administration of 4 to 6 mgm. of  $\alpha$  estradiol benzoate to 4 women did not affect mannitol or PAH clearances or glucose or PAH Tm, but ascorbic acid Tm was diminished (15). When  $\alpha$  estradiol benzoate is given to female dogs the renal load of ascorbic acid necessary to achieve Tm is increased, but with sufficiently increased load normal ascorbic acid Tm values are still obtained; creatinine clearance is slightly increased. Tubular reabsorption of ascorbic acid at low and moderate plasma levels is decreased, increasing clearance (16). Large doses of thyroid by mouth, or thyroxin 20-40 mgm. daily to normal female dogs, increase creatinine clearance and glucose and D Tm (17). Thyroid administration (5 grams daily) increases creatinine clearance in normal, latent polyuric and polyuric dogs to about the same degree, although the increase in daily urine output is much greater in the two latter groups; it raises glucose Tm and diminishes the anti-diuretic effect of pituitrin (18).

The present paper reports observations on D and inulin plasma clearances and on D Tm in female dogs as affected by thyroidectomy, by ovariectomy and by adrenal insufficiency; the effects of various endocrine administrations on some of these animals were also followed. Some observations of the effect of simple hypophysectomy on cardiac output are also given. The procedures for carrying out the clearances and Tm and the chemical methods used have been noted (9); serum sodium was determined according to Butler and Tuthill (19), chlorides according to Sendroy (20), cholesterol by a modification of Bloor's method (21) and nonprotein nitrogen by a micro Kjeldahl with direct Nesslerization.

*Thyroidectomy.* Observations were made on 2 dogs before operation and at intervals thereafter, up to 13 months, including periods of thyroid (Parke, Davis and Co.) feeding and of anterior lobe extract<sup>4</sup> administration. The results are given in table 1.

There is seen to be a small but probably significant fall in D plasma clearance, the values about 8 months after thyroidectomy being 86 and 75 per cent of normal in the 2 cases and remaining there for the several additional months of observation. Inulin plasma clearance is not significantly changed, although K34 may show some decrease. Diodrast Tm falls significantly but later rises so that a year after operation it is only slightly below the preoperative value; the effect is far less than that produced by hypophysectomy. Thyroid administration (0.1 gram/kilo/day orally) to these thyroidectomized dogs restored D plasma clearance to normal, had but little effect on inulin clearance and significantly raised D Tm. The effect on D clearance is greater than it is in normal dogs and less

<sup>4</sup> This extract, Preloban, and the diodrast were given us by the Winthrop Chemical Company.

than in hypophysectomized dogs; the effect on inulin clearance compares with that in normal dogs and is less than in hypophysectomized dogs; and the effect on D Tm is about the same as in normals but less than in hypophysectomized dogs (11). It will be noted that the dose of thyroid used here and in (11) is about  $\frac{1}{3}$  to  $\frac{1}{4}$  of that found by other workers (17) (18) to produce larger increases in creatinine clearance and in glucose and D Tm.

TABLE 1  
*Effect of thyroidectomy on some renal functions*

dog K30				dog K34			
Date	D clearance	Inulin clearance	D Tm	Date	D clearance	Inulin clearance	D Tm
	cc./min./M <sup>2</sup>	cc./min./M <sup>2</sup>	mgm. I/min./M <sup>2</sup>		cc./min./M <sup>2</sup>	cc./min./M <sup>2</sup>	mgm. I/min./M <sup>2</sup>
12/31/43 normal	376	111.0	22.07	12/15/43 normal	270.0	69.5	22.14
1/ 7/44 normal	397	121.3	25.54	12/23/43 normal	305.9	95.3	23.13
1/11/44—Total thyroidectomy				1/11/44—Total thyroidectomy			
4/ 4/44	317.5	98.2	14.6	3/21/44	275.7	56.8	19.45
9/20/44	331.4	114.2	21.4	6/ 6/44	224.0	78.3	14.52
10/ 3/44 through 10/11/44—0.1 gram desiccated thyroid/kilo/day—orally				9/13/44	216.4	84.4	17.61
10/12/44	392.0	100.8	30.7	9/19/44 through 9/27/44—0.1 gram de- desiccated thyroid/kilo/day—orally			
12/ 8/44	331.1	101.8	21.6	9/28/44	285.5	90.6	22.81
12/13/44 through 12/21/44—225 units Preloban/day				1/17/45	217.5	63.0	18.08
12/22/44	366.1	102.1	25.9	1/21/45 through 2/1/45 225 units Prelo- ban/day			
12/27/44 through 1/4/45—225 units Prelo- ban/day				2/ 2/45	320.5	96.1	19.84
1/ 5/45	332/4	89.7	25.96				

Anterior lobe administration had no significant effect on D or inulin clearance in K30; there was a possibly significant rise in D Tm. In K34 it produced significant rises in D and inulin plasma clearances, with no significant effect on D Tm. These inconsistent effects of anterior lobe administration to thyroidectomized dogs may be compared with the consistently large increases in D and inulin clearances and in D Tm produced by anterior lobe administration to normal and to hypophysectomized dogs (11), indicating that a considerable part of the response of normal and of hypophysectomized dogs to anterior lobe administration may

be due to its thyrotropic action, or that the presence of thyroid hormone may abet the action of a postulated "renotropic" hormone.

*Ovariectomy.* Observations were made on 2 dogs before bilateral ovariectomy, and at intervals after operation up to about a year. The operation was without effect on D or inulin clearances or on D Tm. The results are given in table 2.

*Adrenal insufficiency.* In 5 female dogs an attempt was made to produce chronic and progressive adrenal insufficiency by wrapping the left adrenal in silk and subsequently removing the right adrenal; in only 1 case, K70, was the desired end accomplished. The findings on this dog and on 1 dog, K31, in which the original plan failed to produce adrenal insufficiency and in which removal of the

TABLE 2  
*Effect of bilateral ovariectomy on some renal functions*

dog K25				dog K28			
Date	D clearance	Inulin clearance	D Tm	Date	D clearance	Inulin clearance	D Tm
	cc./min./M <sup>2</sup>	cc./min./M <sup>2</sup>	mgm. I/min./M <sup>2</sup>		cc./min./M <sup>2</sup>	cc./min./M <sup>2</sup>	mgm. I/min./M <sup>2</sup>
11/26/43 normal	225.8	82.3	11.91	4/ 8/43 normal	221.1	73.1	22.41
12/ 8/43—bilateral ovariectomy				11/12/43 normal	250.1	66.9	26.38
4/ 9/44	218.7	70.4	14.80	12/ 8/43—bilateral ovariectomy			
10/ 5/44	202.4	70.2	12.65	2/ 5/44	225.9	63.3	24.41
10/19/44	199.0	78.2	—	5/31/44	280.5	78.3	22.40
11/ 9/44	212.2	83.9	—	10/25/44	235.6	75.6	27.32

second adrenal was finally carried out, are shown in table 3. Evidence for development of adrenal insufficiency was obtained by observing food intake and general condition and by following plasma nonprotein nitrogen. No preoperative observations were obtained on K70.

On 4/5/44 K 70 showed definite signs of adrenal insufficiency; the D and inulin clearances and D Tm were far below average normal values.

Administration of adrenal cortical extract (Upjohn) (ACE) raised inulin clearance but was without effect on D clearance and D Tm. Continued administration of cortical extract plus desoxycorticosterone acetate in oil (Doca) (Roche Organon, Inc.) raised D clearance and still further raised inulin clearance but D Tm remained unchanged. Since the beginning of replacement therapy on 4/5/44, after the first set of observations, the dog had appeared to be in good condition, had good food intake and normal non-protein nitrogen and was quite active; there is no knowledge of the dog's general condition and food intake after the experiment of 4/26/44; she was found dead on the morning of 4/27/44.

TABLE 3

*Effects of adrenal insufficiency on some renal functions*

REMARKS	D CLEARANCE	INULIN CLEARANCE	D Tm	PLASMA NPN	PLASMA Cl	PLASMA CHOLE- STEROL
Dog K70						
	cc./ min./M <sup>2</sup>	cc./ min./M <sup>2</sup>	mgm. I/ min./M <sup>2</sup>	mgm./ 100 cc.	mEq./L.	mgm./ 100 cc.
10/20/43 Left adrenal wrapped in silk 1/ 3/44 Right adrenalectomy 4/ 5/44 Dog not eating well, weak	188	42.5	5.69	61	117	98
4/ 5/44 Through 4/17/44 5 cc. ACE twice daily 4/13/44	179	63.8	5.41	29		139
4/18/44 Through 4/26/44 1 mgm. Doca in mornings; 2 cc. ACE in afternoons. Daily food intake good 4/26/44	244	72.8	5.79	24	123	100
4/27/44 Dog found dead in cage in morning						
Dog K31						
					PLASMA Na	
2/ 3/44 Normal 2/ 9/44 Normal	267 291	89.5 73.2	21.4 23.3			151
2/11/44 Left adrenal wrapped in silk 3/ 1/44 Right adrenalectomy 5/ 5/44 7/13/44 9/ 7/44	325 315 373	103 94.6 110	15.5 14.1 20.1	24 41 28	146	233 104
5/18/45 Plasma volume 44.5 cc./ kilo; blood volume 91.2 cc./kilo 5/23/45	296	76.1	19.6	30	146	153
6/ 5/45 Left adrenalectomy 6/ 4/45 Through 6/17/45 6 mgm. Doca and 150 units Pre- loban in mornings; 4 cc. ACE in afternoons 6/ 8/45 6/12/45	286	91.5	20.1	29 22		
6/18/45 Through morning of 6/22/45 150 units Preloban in mornings; Doca and ACE discontinued 6/19/45 6/20/45 6/21/45 6/22/45 Plasma volume 43.8 cc./ kilo; blood volume 86 cc./kilo;	197	94	7.3	22 31 42 52	134	

TABLE 3—Concluded

REMARKS	D CLEARANCE	INULIN CLEARANCE	D Tm	PLASMA NPN	PLASMA Cl	PLASMA CHOLESTEROL
	cc./ min./M <sup>2</sup>	cc./ min./M <sup>2</sup>	mgm. I/ min./M <sup>2</sup>	mgm./ 100 cc.	mEq./L.	mgm./ 100 cc.
8 cc. ACE and 6 mgm. Doca after experiment 6/23/45 Through 6/30/45 6 mgm. Doca and 150 units Pre- loban in mornings; 4 cc. ACE in afternoons 6/28/45	182	79	5.15	30		
7/ 1/45 Through 7/9/45 6 mgm. Doca and 4 cc. ACE daily; Preloban discon- tinued 7/ 6/45	221	70.4	8.63	31		
7/10/45 Through 7/14/45 12.5 mgm. Doca and 8 cc. ACE daily 7/14/45	179	71.7	4.31	37	147	108
7/15/45 Through 7/20/45 12.5 mgm. Doca and 8 cc. ACE in mornings; 150 units Pre- loban in afternoons 7/20/45	197	62.7	8.12	50		
7/21/45 Through 7/25/45 6 mgm. Doca and 4 cc. ACE daily 7/25/45 4 pellets of desoxycortico- sterone acetate (125 mgm. /pellet) implanted sub- cutaneously 7/26/45 Through 7/28/45 2 mgm. Doca and 2 cc. ACE daily 8/ 4/45 9/28/45	263	74	13.8	29 34		63
10/ 8/45 Through 10/26/45 225 units Preloban daily 10/16/45 Dog went into collapse during D Tm, blood sugar 25 mgm./100 cc.	308	111		22	147	55
10/26/45 450 cc. 5% glucose orally at beginning of experi- ment. Dog went into collapse during attempted D Tm 1/ 6/46 165 days after implanting pellets dog died; no trace of pellets found at autopsy						

Dog K31 failed to develop any evidence of adrenal insufficiency after wrapping of left adrenal and removal of right; she was followed for 15 months and continued to appear normal and to show normal blood chemistry. During this period D and inulin clearances and D Tm remained normal, except for a temporary fall in D Tm. On 6/5/45 the second adrenal was removed and the dog maintained as shown in table 3. On 6/12/45, while the dog was being maintained on Doca and ACE, with Preloban also given, the values remained normal. On 6/18/45 adrenal replacement therapy was discontinued, with Preloban administration continuing. On 6/22/45, after 5 days without replacement therapy, there was definite evidence of adrenal insufficiency, and D clearance was somewhat reduced, with a marked reduction in D Tm, while inulin clearance remained normal. This shows, as would be expected, that anterior lobe administration does not check the development of adrenal insufficiency in the adrenalectomized dog. It shows further that in adrenal insufficiency anterior lobe extract cannot elevate D clearance and D Tm, as it does in the normal and in the hypophysectomized dog (11). On 6/22/45, after the clearance experiment, adrenal replacement therapy was again begun, with Preloban continued, continuing through 6/30/45; values remained depressed on 6/28/45. From 7/1/45 through 7/14/45 adrenal therapy was continued, without Preloban administration; values remained depressed on 7/6/45 and 7/14/45. From 7/15/45 through 7/20/45 Preloban was again added to the adrenal replacement therapy; values remained depressed on 7/20/45. It appears that the adrenal replacement therapy afforded by the daily administrations indicated in the table is not adequate to restore the depressed values to normal nor to permit the animal to respond in the normal fashion to anterior lobe administration, although the same or less replacement therapy had sufficed to keep the dog from going into insufficiency.

On 7/25/45 4 pellets of desoxycorticosterone acetate<sup>5</sup> of 125 mgm. each were implanted subcutaneously. On 9/28/45 D clearance and D Tm were definitely raised above the values shown on the daily replacement therapy. From 10/8/45 through 10/26/45 Preloban was added; on 10/16/45 D and inulin clearances were restored to normal; attempts to measure D Tm on this date and on 10/26/45 were unsuccessful. We believe that D Tm values on 10/16/45 and on 10/26/45 would have been as high as or higher than on 9/28/45. These findings show that subcutaneously implanted desoxycorticosterone acetate pellets afford adequate replacement therapy for the adrenalectomized dog, whereas daily subcutaneous administration of Doca and of ACE, with or without Preloban added, did not, so far as D and inulin clearances and D Tm values are concerned. As the pellets were depleted the dog developed adrenal insufficiency and died unexpectedly on 1/6/46, no further observations being made during this second and final period of insufficiency.

These observations show that a sufficient degree of adrenal insufficiency lowers D and inulin clearances and D Tm as much as does hypophysectomy; indeed, there is no reason to doubt that observations during the terminal phases of insufficiency would show even greater depression than is seen in hypophysectomized animals.

<sup>5</sup> These pellets were given us by Ciba Pharmaceutical Products, Inc.

Anterior lobe administration did not raise the renal functions under discussion during adrenal insufficiency (table 3, K31, 6/22/45) nor during the replacement therapy afforded by daily administration of Doca and ACE (table 3, 6/28/45 and 7/20/45), but did during more adequate replacement therapy by pellets (table 3, 10/16/45). Daily administration of ACE did not raise these functions in hypophysectomized dogs (11). This raises the question as to whether adequate adrenal replacement therapy, by pellets, would restore these functions in the hypophysectomized dog; this experiment has not yet been done.

*Effect of simple hypophysectomy on cardiac output.*<sup>6</sup> The effect of simple hypophysectomy on cardiac output has been determined in a series of 5 dogs. Cardiac output measurements were made by the Fick method on dogs under sodium pentobarbital. Mixed venous blood was obtained by right ventricular puncture

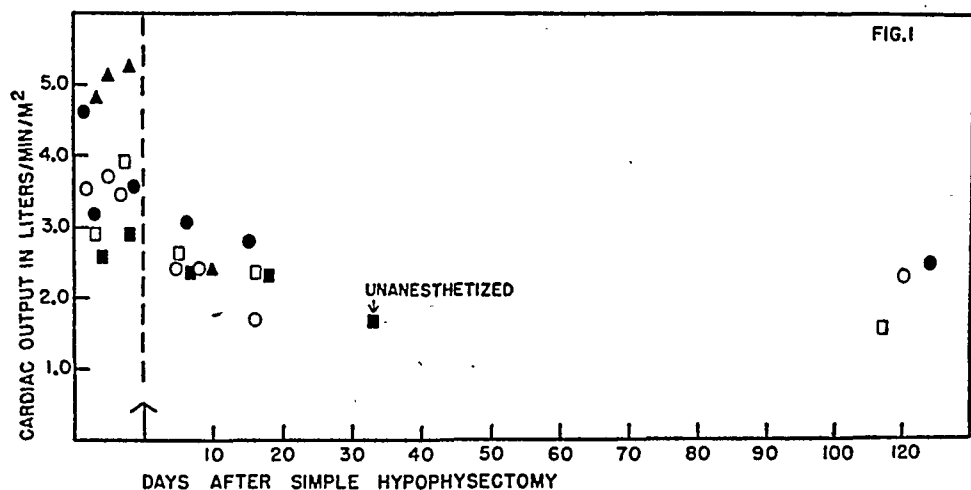


Fig. 1. Effect of hypophysectomy on cardiac output.

through the chest wall, arterial blood by puncture of the femoral artery. Blood samples were drawn under oil with syringes and oxygen content determined in duplicate with the Van Slyke manometric apparatus. Rate of oxygen consumption was determined by a modified small Roth-Benedict apparatus, using a face mask. In all cases determinations were made before operation and at intervals after simple hypophysectomy. The results are seen in figure 1.

Within a few days after simple hypophysectomy, cardiac output falls to 50–80 per cent of normal, with usually a further fall so that in about 3 weeks it is 50–60 per cent of normal. There is no further fall, at least up to 4 months.

**DISCUSSION.** It is recognized that, due to the time-consuming nature of these experiments, the number of observations is not as great as seems desirable. On some points the results are sufficiently clear cut to permit definite conclusions; on others they are merely suggestive. Table 4 gives a resumé of the directional changes found.

There is no doubt that (a) hypophysectomy, simple or total, consistently pro-

<sup>6</sup> Dr. H. W. Fischer of the Department of Surgery aided us in some of these experiments.

duces large falls in D and inulin clearances and in D Tm, the last showing the greatest percentage fall; (b) the fall in D clearance means fall in renal plasma flow, since renal extraction of D is not changed; (c) the falls come on within a few days after hypophysectomy and are permanent. The findings of figure 1 of the present paper show that cardiac output also falls, and to about the same degree as

TABLE 4

*A resumé of the effects of certain endocrine influences on D and inulin clearances and on D Tm in the dog*

1	2	3 NONE	4 ANTERIOR LOBE	5 THYROID	6 ACE	7 ACE PLUS DOCA	8 DESOXYCORTI- COSTERONE ACETATE PELLETS
Normal	D clearance Inulin clearance D Tm	Normal Normal Normal	Increase Increase Increase	Increase Increase Increase	No effect No effect or increase No effect		
Hypophy- sectomy, simple or total	D clearance Inulin clearance D Tm	Decrease Decrease Decrease	Increase Increase Increase	Increase Increase Increase	No effect No effect No effect		
Puncture	D clearance Inulin clearance D Tm	Normal or increase Normal Normal or increase	Increase Increase Increase	Increase Increase Increase	No effect or increase No effect No effect		
Thyroid- ectomy	D clearance Inulin clearance D Tm	Decrease Normal or decrease Decrease	No effect or slight increase	Increase No effect or increase Increase			
Ovariectomy	D clearance Inulin clearance D Tm	Normal Normal Normal					
Adrenal in- sufficiency	D clearance Inulin clearance D Tm	Decrease Decrease Decrease	No effect No effect No effect		No effect Increase No effect	Prevents further decrease	Increase Increase Increase

The effects on D and inulin clearances and on D Tm of various operations on the endocrine system, and of the endocrine administrations of the top horizontal line to the different types of dogs in column 1 are shown; the effects designated in columns 4 to 8 are with respect to the conditions in column 3. This table cannot present all the facts, as variations with time and the different magnitudes of response to various procedures. In general, it shows the directional changes of the late effects.

renal blood flow. Fall in rate of oxygen consumption approximately equalled fall in cardiac output.

The renal functions studied here have been taken both because of inherent interest in endocrine influences on the kidney and because they afford a means for quantitative measurements of the degree of various endocrine deficiencies and replacements and their interrelationships. Some questions are answered and others are raised by the findings in hypophysectomized animals. The fact that the findings are essentially the same after simple as after total hypophysectomy

shows that loss of neurohypophysis is not of primary importance. We may also be sure that loss of thyrotropic hormone is not the principal reason for the fall in these values in the hypophysectomized dog, since the falls resulting from total thyroidectomy are much less than those seen with hypophysectomy. On the other hand, the presence of thyroid hormone does play a part; this is further shown by the findings that anterior lobe administration, capable of causing large increases in these values in the normal and in the hypophysectomized dog, has little or no effect on the thyroidectomized dog, and that thyroid administration raises the values in normal, in hypophysectomized and in thyroidectomized dogs. We can say further that the falls in these values seen after hypophysectomy are not due to loss of gonadotropic hormone, since the values are unchanged by ovariectomy.

The possibilities remain that the falls in these renal functions seen on hypophysectomy are due (a) mainly to loss of adrenotropic hormone; (b) that they are due to loss of a hormone which itself acts on the kidney ("renotropic"), or (c) that they are due to the overall changes in the organism's metabolism. The last suggestion does not convey much meaning and, in any event, it is desirable to attempt to consider the various component processes in the overall metabolic changes. The falls in renal functions seen after hypophysectomy cannot be due merely to the depression of oxygen consumption, since they are much greater than after thyroidectomy, although depression of oxygen consumption after thyroidectomy is essentially as great as after hypophysectomy.

The data do not permit a decision between loss of adrenotropic hormone and of a "renotropic" hormone, the latter term being used in the sense of a substance formed in the pars distalis, essential to the normal maintenance of renal blood flow, glomerular filtration rate and tubular capacity to transport D, without commitment as to the mechanisms involved, which does not act through the mediation of other endocrine glands, but which may require the presence of some adrenal cortical hormone in order to be effective. In mild to moderate adrenal insufficiency the above renal functions fall as much as they do with hypophysectomy. If it could be shown that the adrenals of the hypophysectomized animals are not sufficiently depressed to account for our renal findings, the idea of a "renotropic" hormone would be supported. We know that hypophysectomized dogs are not in a severe adrenal insufficiency, since they will live for years without therapy and continue to show normal plasma sodium and potassium levels and only slight to moderate elevation in plasma nonprotein nitrogen (table 5). Nevertheless, it is certain that their adrenal cortical function is not normal. The findings on K31, table 3, 6/28, 7/6 and 7/14/45, during daily adrenal replacement therapy and while blood volume and plasma nonprotein nitrogen and sodium are normal, show that a very mild degree of asymptomatic adrenal insufficiency may be sufficient to depress D and inulin clearances and D Tm; these animals are presumably comparable with asymptomatic intercritical Addison's patients (22) and probably show no more adrenal deficiency than do hypophysectomized dogs. The question may legitimately be raised, however, as to whether depression of adrenal cortical function within the first few days after

hypophysectomy can be great enough to account for the depressions in clearances and Tm observed at that time, even though it be granted that only a slight degree of insufficiency may suffice to depress such clearances and Tm. If it is concluded

TABLE 5

*Plasma NPN, sodium and potassium findings in normal and operated dogs*

REMARKS	PLASMA NPN	PLASMA Na	PLASMA K
	<i>mgm./ 100 cc.</i>	<i>mEq./L.</i>	<i>mEq./L.</i>
Normal dogs	24	146	4.86
	29	147	4.83
	33	146	
	24	144	
	28	145	
K16 (total hypophysectomy) 137 days post. op. 205 days post. op.	54	143	5.78
K17 (total hypophysectomy) 161 days post. op. 219 days post. op.	51	143	5.45
AI (total hypophysectomy) 211 days post. op. 290 days post. op.		147	
		148	
K21 (simple hypophysectomy) 91 days post. op. 159 days post. op.	63	140	4.91
K22 (simple hypophysectomy) 51 days post. op. 119 days post. op.	55		
		141	5.55
No. 3 (simple hypophysectomy) 226 days post. op.	43		
No. 2 (simple hypophysectomy + thyroidectomy) 225 days after hypophysectomy; 72 days after thyroidectomy	52		
No. 4 (simple hypophysectomy + thyroidectomy) 225 days after hypophysectomy; 72 days after thyroidectomy	47		
K20 (puncture) 119 days post. op. 187 days post. op.	53		
		143	5.32
K18 (puncture) 70 days post. op. 148 days post. op. 5 yrs., 10 mos. post. op.	45		
	30	145	5.40
No. 8 (thyroidectomy) 59 days post. op.	32		

that the observed depressions, early as well as late, of renal functions with hypophysectomy are due primarily to depression of adrenal cortical function, it follows that a relatively slight depression of adrenal function suffices for maximum ob-

served depression of the renal functions under discussion, since these functions have fallen to their permanently low values within 3 or 4 weeks after hypophysectomy, while adrenal cortical regression continues much longer. It seems probable that our observed depressions in D and inulin clearances and in D Tm in hypophysectomized dogs may be ascribed to loss of adrenotropic hormone with resultant adrenal insufficiency, although the concept of a "renotropic" hormone or of a synergism between such postulated hormone and an adrenal cortical hormone cannot be excluded.

#### SUMMARY

Thyroidectomy in dogs produces some fall in D clearance and D Tm, with little or no effect on inulin clearance; the effects are far less than those of hypophysectomy. Thyroid administration to thyroidectomized dogs raises D clearances and D Tm. Anterior lobe administration which gives large increases in these clearances and in D Tm in the normal or the hypophysectomized dog produces small and inconsistent rises in the thyroidectomized dog.

Bilateral ovariectomy does not affect D or inulin clearances or D Tm.

Very mild adrenal insufficiency, asymptomatic and with normal blood volume and nonprotein nitrogen, sodium and potassium plasma levels, may cause large falls in D clearance and D Tm, with smaller fall in inulin clearance. Values were restored to normal by implantation of pellets of desoxycorticosterone acetate, which were more effective than was daily subcutaneous administration of Doca and ACE. The asymptomatic adrenal deficient dog does not respond to anterior lobe administration with increases in clearances and D Tm, as does the normal or the hypophysectomized dog.

The falls in clearance and Tm values seen in hypophysectomy are not due to loss of thyrotropic or of gonadotropic hormone, nor to the reduction in oxygen consumption *per se*. The question of whether the effects can be ascribed mainly to loss of adrenotropic hormone or whether some other deficiencies are operative is left unanswered.

The cardiac output is greatly and permanently reduced after hypophysectomy, and to about the same degree as are oxygen consumption and renal blood flow.

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# ALKALINE PHOSPHATASE LEVELS IN PLASMA AND LIVER FOLLOWING PARTIAL HEPATECTOMY

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Information on the behavior of alkaline phosphatase following partial hepatectomy is very meager. Freeman, Chen and Ivy (1) noted a small and transient increase in the plasma phosphatase of two dogs following removal of 30 per cent of the liver. Greenstein (2) found no change in alkaline phosphatase of the liver of the rat following removal of 70 per cent of that organ. During the restoration of liver following partial hepatectomy in the rat there is an increased turnover of such phosphate compounds as the phospholipids (3) and nucleic acids (4) in the liver. This increase might be associated with the activity of some type of phosphatase. If some relation of phosphatase to restoration of liver following partial hepatectomy (5) could be demonstrated it might throw some light on the origin and importance of alkaline phosphatase. Mann (6) has pointed out that no changes in function have been noted in the regenerating liver.

**METHODS.** Male rats of the Sprague-Dawley strain, weighing approximately 200 to 300 grams, were used. While the animals were under ether anesthesia, the large median lobe and the right lateral lobe, which make up approximately 70 per cent of the liver, were separately tied near the hilus of the liver and removed.

The rats were returned to their cages and given free access to a commercial diet (Friskies) and water. Since the level of alkaline phosphatase is influenced by amount and composition of food intake (7-9) and the rate (10) and degree (11) of restoration of hepatic tissue is affected by diet, the forty-eight hourly post-operative food intake of the rats was recorded.

At intervals of one, two, three, four, seven, nine and fifteen days thereafter blood was obtained by cardiac puncture with the rats under light ether anesthesia and analysis for alkaline phosphatase activity was performed. Minimal amounts of heparin were used to prevent coagulation. After blood samples had been drawn, some rats were killed to obtain residual hepatic weights while others were allowed to recover to be used for further determinations of alkaline phosphatase.

Plasma phosphatase was estimated according to the method of Bodansky (12). Inorganic phosphate was determined according to the method of Fiske and Subbarow (13). Careful notation of the presence or absence of bile pigments in the plasma was made.

When the livers were analyzed also, the rats were anesthetized with pentobarbital sodium and the livers were removed and extracted in physiologic saline solution in a Waring Blendor. After centrifugation an aliquot was incubated at

37° for one hour with the sodium veronal buffer containing glycerophosphate, and another aliquot with this buffer only, for inorganic phosphate content. At the end of this interval the proteins were precipitated with trichloroacetic acid and the phosphatase was determined as for plasma.

TABLE 1  
*Effect of fasting on alkaline phosphatase*

TIME OF FASTING	RATS	BODY WEIGHT	LIVER WEIGHT	PLASMA PHOSPHATASE	LIVER PHOSPHATASE
hours		grams	per cent of body weight	Bodansky units per 100 ml.	Bodansky units per 100 grams wet weight
0	15	278 $\pm$ 7*	3.22 $\pm$ 0.08	34.6 $\pm$ 2.1	32.0 $\pm$ 1.6
24	6	205 $\pm$ 7	3.38 $\pm$ 0.14	17.1 $\pm$ 2.2	18.8 $\pm$ 4.0
48	6	202 $\pm$ 10	3.33 $\pm$ 0.14	10.8 $\pm$ 1.3	6.5 $\pm$ 1.7

\* The values following the  $\pm$  sign represent the standard error of the mean.

TABLE 2  
*Effect of partial hepatectomy on alkaline phosphatase*

TIME AFTER PARTIAL HEPATECTOMY	RATS	BODY WEIGHT	LIVER WEIGHT	PLASMA PHOSPHATASE	LIVER PHOSPHATASE
days		grams	per cent of body weight	Bodansky units per 100 ml.	Bodansky units per 100 grams wet weight
1	16	212 $\pm$ 9*	1.82 $\pm$ 0.05	56.5 $\pm$ 3.9	93.8 $\pm$ 12.7
2	6	200 $\pm$ 5	2.06 $\pm$ 0.43	78.9 $\pm$ 4.1	106.8 $\pm$ 11.6
3	6	199 $\pm$ 6	2.59 $\pm$ 0.07	74.0 $\pm$ 2.4	73.9 $\pm$ 4.0
4	6	217 $\pm$ 5	3.20 $\pm$ 0.15	54.2 $\pm$ 6.4	61.8 $\pm$ 11.1
7-9	9	200 $\pm$ 3	3.53 $\pm$ 0.16	46.3 $\pm$ 5.1	39.2 $\pm$ 2.4
15	6	316 $\pm$ 10	3.04 $\pm$ 0.07	36.7 $\pm$ 2.8	35.7 $\pm$ 1.7

\* The values following the  $\pm$  sign represent the standard error of the mean.

TABLE 3  
*Effect of laparotomy on alkaline phosphatase*

TIME AFTER LAPAROTOMY	RATS	BODY WEIGHT	LIVER WEIGHT	PLASMA PHOSPHATASE	LIVER PHOSPHATASE
days		grams	per cent of body weight	Bodansky units per 100 ml.	Bodansky units per 100 grams wet weight
1	6	199 $\pm$ 2*	3.72 $\pm$ 0.11	21.4 $\pm$ 2	32.6 $\pm$ 2
2	2	240, 260	3.04, 3.76	38.6 $\pm$ 6.2†	24.8, 27.1
4	8	223 $\pm$ 7	3.37 $\pm$ 0.09	24.9 $\pm$ 2.8	22.8 $\pm$ 1.4
9	2	188, 204	3.93, 5.60	23.5, 28.8	24.5, 43.8

\* The values following the  $\pm$  sign represent the standard error of the mean.

† This mean is based on observations on four rats.

In several cases of each group laparotomy was performed but no hepatic tissue was removed. These served as controls for the operative effect. A sufficient number of normal rats on which operation was not performed were also included to estab-

blish the control values for phosphatase in this group. The effect of fasting was studied in the normal rats since the food intake of the rats that underwent operation was variable for a day or two after the operation.

**RESULTS.** A progressive decrease of the level of alkaline phosphatase in both plasma and liver was noted following a fast of one or two days (table 1). An increase of the mean value for the plasma phosphatase was found as early as twenty-four hours after partial hepatectomy and this increase was quite independent of the amount of food eaten during this interval (table 2). The peak of elevation of the level of phosphatase came on the second and third days. There was a gradual decrease after the fourth day, with normal values present in most rats in two weeks. In four rats weighing about 350 grams in which only the plasma phosphatase was determined a mean value of  $69.7 \pm 4.6$  Bodansky units per 100 ml. was found at two weeks. Normal values were found in rats following laparotomy (table 3).

The presence of icteric plasma was in no way correlated with elevation of plasma phosphatase. This was true whether different rats were compared or the same animal was studied at different intervals.

The highest values for alkaline phosphatase of the liver were found at one and two days after partial hepatectomy with a gradual decrease to normal values in nine days. Liver and plasma phosphatase did not differ by more than 7 or 8 units except on the first two days after partial hepatectomy when the difference was as much as 37.3 units, the value for liver being higher than that for plasma (table 2).

**COMMENT.** It has been shown that after removal of 70 per cent of the hepatic substance, there is an increase in size of cells during the first day, with the maximal rate of restoration of hepatic parenchyma occurring from forty-eight to seventy-two hours (5, 10). This is also the period of greatest mitotic activity (5, 14). The rise of both plasma and liver phosphatase during this interval may mean that rapidly growing hepatic cells produce larger amounts of phosphatase than normal hepatic cells. Higher levels than these have been found in fetal liver and much higher levels in hepatomas (2, 15). Plasma values lag behind liver during the first two days but catch up and remain very close thereafter (table 2).

Considering the great difference in normal values for plasma phosphatase in the dog and rat, the elevation found in our rats might be considered roughly comparable to that found by Freeman, Chen and Ivy (1) in dogs after removal of about 33 per cent of the liver. The higher percentage of liver removed in our case probably accounts for the longer persistence of the elevated plasma phosphatase after 70 per cent partial hepatectomy in rats.

No elevation of alkaline phosphatase in regenerating liver was found, however, by Greenstein (2), who removed 70 per cent of the liver from rats and studied the remaining portion of the liver at two and three days after hepatectomy. His measurements of hepatic phosphatase were made after an eighteen hour period of autolysis, while ours were made as soon as possible after the tissue extracts were prepared.

Our findings agree with those of others in that the elevated plasma phosphatase need not correlate with bile pigment (1, 16). Likewise our rats showed the usual response to fasting in their plasma phosphatase. Hepatic phosphatase decreased in a similar manner with fasting.

Higgins and Anderson (5) have pointed out that the liver is fully restored ten to fourteen days after 70 per cent partial hepatectomy in rats. This agrees well with the time at which our liver and plasma levels of alkaline phosphatase have returned to normal. This fact, in conjunction with the finding that the period of maximal rate of restoration and appearance of mitotic figures corresponds to that of the highest levels of alkaline phosphatase in the plasma and the liver, tends to support the hepatic origin of this enzyme under the conditions of these experiments.

#### CONCLUSIONS

1. Alkaline phosphatase is elevated in the plasma and the liver after 70 per cent partial hepatectomy in rats.

2. Elevations of alkaline phosphatase in the liver are greater than those found in plasma on the first two days after 70 per cent partial hepatectomy. From the third day on, levels in the plasma and in the liver agree closely.

3. Evidence is presented in support of the hepatic origin of alkaline phosphatase after partial hepatectomy in rats.

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# THE EFFECT OF HEMORRHAGE AND MUSCLE TRAUMA UPON THE BLOOD PHOSPHATE OF DOGS<sup>1</sup>

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Recent studies made in this laboratory have shown that the decrease in the blood volume of the dog following muscle trauma or hemorrhage (1) is associated with a slowed blood flow (2) which produces certain changes in the blood electrolytes (3). Among the latter is an increase in the plasma inorganic phosphate concentration. The present investigation has been undertaken to examine in detail the rise in phosphorus which follows muscle trauma and to compare this with the increase which occurs after hemorrhage. In addition, we have studied the relation of the plasma phosphatemia to the phosphorus and chloride contents of the red blood cells.

**METHODS.** The experiments were done on mongrel dogs. The animals were not fed after noon of the day preceding the experiment, but were permitted to drink water freely.

**Trauma.** The thigh muscles were contused according to the method of Gregeresen and Root (1). After producing the desired injury which took some 15 to 20 minutes, the administration of ether was discontinued.

**Hemorrhage.** Hemorrhage was carried out in the manner described by Walcott (4). The method involves bleeding the animal through a cannula inserted in the femoral artery under local anesthesia (2 per cent novocain). When the bleeding is virtually complete, some fraction of the volume of blood obtained is immediately returned to the circulation. According to the terminology used in this method, a 75 per cent hemorrhage means that 25 per cent of the blood removed has been returned to the circulation immediately after bleeding was complete.

**Blood volume** was calculated from the plasma volume as determined with the blue dye, T-1824, and the hematocrit reading (1,5).

The *pH* of whole blood was determined at 38°C. immediately after withdrawal using a hypodermic type of the McInnes glass electrode (6).

**Phosphorus.** The colorimetric method of Fiske and Subbarow (7) as modified by Guest and Rapoport (8) was used for phosphorus estimation. Amidol was substituted for amino naphthol sulfonic acid as the reducing agent (9). Plasma and whole blood phosphorus were determined directly. Cell phosphorus was calculated from the whole blood and plasma determinations and the hematocrit readings.

<sup>1</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Columbia University.

*Chloride.* Blood chlorides were determined by the Van Slyke method (10).

*O<sub>2</sub> content* of jugular venous blood was estimated by the method of Van Slyke and Neill (11).

*Water.* The percentage of water in blood and plasma was obtained by drying the samples at 78°C. to a constant weight. The cell water was calculated from these values.

**RESULTS:** The progressive increases in concentration of hydrogen ions and inorganic phosphorus in the blood of dogs developing fatal shock after severe muscle trauma are illustrated by figure 1 in which time is plotted against blood pH and mM. of inorganic phosphorus per liter of plasma. A similar experiment in which the blood volume was reduced by hemorrhage is shown in figure 2. In

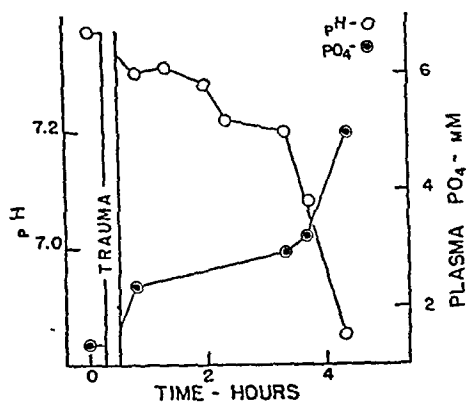


Fig. 1

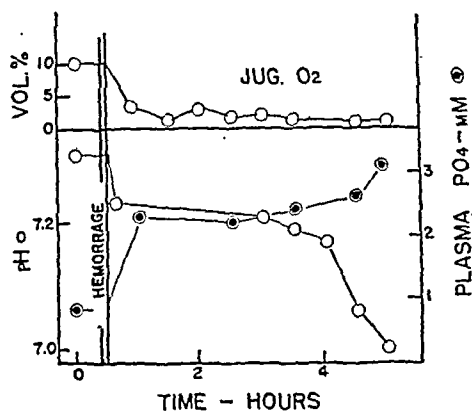


Fig. 2

Fig. 1. The effect of muscle trauma upon the plasma phosphorus and the blood pH. Time in hours is plotted along the abscissa; blood pH and plasma phosphorus in mM. per liter of plasma, along the ordinate.

Fig. 2. The effect of hemorrhage upon the plasma phosphorus, the blood pH and the jugular venous O<sub>2</sub> content. Time in hours is plotted along the abscissa. Separate ordinate values are shown for blood pH, jugular venous O<sub>2</sub> in vols. per cent and plasma phosphorus in mM. per liter of plasma.

this figure the development of hypoxia is indicated by the curve of jugular venous O<sub>2</sub> content. Thirty to 40 per cent reduction of the blood volume by either muscle trauma or hemorrhage is followed by a sudden rise in plasma inorganic phosphorus and hydrogen ion concentration which over a period of some hours slowly increases. Immediately before death there is an abrupt rise in the concentration of phosphorus and a sudden fall in pH.

The influence of the severity of hemorrhage upon the concentration of plasma inorganic phosphorus at various times after reduction of the blood volume is shown in figure 3. The curves marked I and II represent the average results obtained from 5 dogs following a 50 per cent hemorrhage and 2 dogs after a 63 per cent hemorrhage, respectively. The curves which terminate at 1½, 2½, 4½ hours (III, IV, V) are, respectively, the averages of 3, 8 and 4 dogs, each of which was subjected to a 75 per cent hemorrhage. The animals died shortly after the

times indicated by the last points on the curves. Curves III, IV and V illustrate the spread which may be expected after a reduction of blood volume of this magnitude. In short, figure 3 shows that the rate of increase in the concentration of plasma phosphorus is a function of the severity of the hemorrhage.

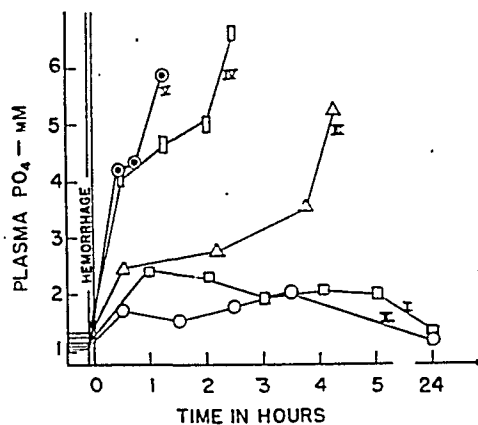


Fig. 3

Fig. 3. The relation of the elapsed time after hemorrhage and the magnitude of the hemorrhage to the plasma phosphorus in mM. per liter of plasma. Curve I is the average of 5 dogs following a 50 per cent hemorrhage. Curve II represents the average of the results obtained on 2 dogs after a 63 per cent hemorrhage. Curves III, IV and V represent different groups of dogs in which death occurred at  $1\frac{1}{2}$ ,  $2\frac{1}{2}$ ,  $4\frac{1}{2}$  hours after a 75 per cent hemorrhage. Curve III is the average of 3; curve IV, of 8; and curve V, of 4 dogs.

Fig. 4. The relation of plasma pH to plasma phosphorus (mM. per liter of plasma) following hemorrhage.

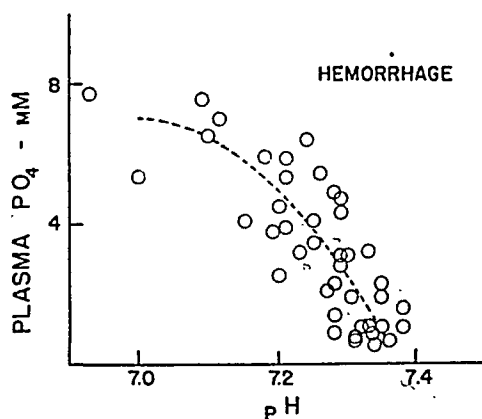


Fig. 4

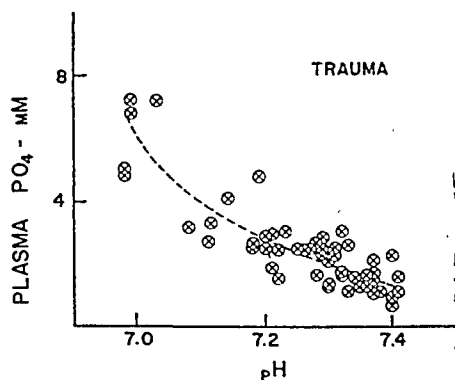


Fig. 5

Fig. 5. The relation of plasma pH to plasma phosphorus (mM. per liter of plasma) following muscle trauma.

Fig. 6. The relation of plasma phosphorus (mM. per liter of plasma) to cell phosphorus (mM. per liter of cells) after hemorrhage and muscle trauma.

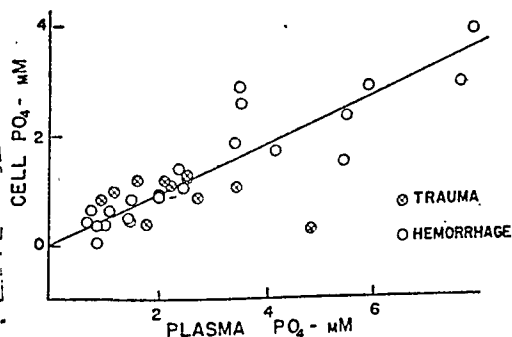


Fig. 6

The relation between blood pH and the concentration of plasma inorganic phosphorus (mM. per liter of plasma) following hemorrhage or muscle trauma is shown in figures 4 and 5. The slopes of the curves differ, plasma phosphorus

increasing more rapidly with decreasing pH (between 7.4 and 7.1) after hemorrhage than after trauma. However, when the pH had decreased to 7.0 which was always near the terminal stage, there was no significant difference between

TABLE 1

*Distribution of inorganic and organic acid-soluble phosphorus between plasma and cells before and after hemorrhage or muscle trauma*

DOG NO.	CONDITION	TIME	pH	RED CELLS	PLASMA INORGANIC P	RED CELLS	
						Inorganic P	Organic P
				%	mgm. %	mgm. %	mgm. %
1	Control	10:52 a.m.	7.30	51.0	2.3	2.0	49.2
	Hemorrhage	12:00-12:11 p.m.					
	Post-hem.	1:36 p.m.	7.21	40.4	16.6	4.8	51.2
	Post-hem.	4:14 p.m.	6.93	44.5	24.1	12.3	48.5
2	Control	11:05 a.m.	7.32	50.5	2.4	1.4	37.3
	Hemorrhage	11:40-54 a.m.					
	Post-hem.	2:17 p.m.	7.25	47.9	12.8	5.5	49.9
	Post-hem.	4:07 p.m.	7.18	44.1	18.0	9.1	60.5
3	Control	10:40 a.m.	7.38	62.9	3.3	1.9	30.5
	Hemorrhage	11:10-11:17 a.m.					
	Post-hem.	11:53 a.m.	7.09	54.9	23.5	9.4	40.8
4	Control	10:20 a.m.	7.40	44.0	2.9	2.7	40.8
	Trauma	11:05-40 a.m.					
	Post-trauma	12:48 p.m.	7.25	49.6	7.8	3.9	26.4
	Post-trauma	2:45 p.m.	7.27	49.5	7.8	4.2	41.2
5	Control	11:09 a.m.	7.37	36.0	3.6	3.1	45.7
	Trauma	11:20-11:30 a.m.					
	Post-trauma	12:35 p.m.	7.32	38.4	5.0	3.7	51.8
	Post-trauma	1:30 p.m.	7.28	38.7	5.0	3.7	51.5
	Post-trauma	2:31 p.m.	7.21	37.3	5.8	2.7	52.6
	Post-trauma	3:30 p.m.	7.12	36.9	8.6	2.7	62.2
	Post-trauma	4:20 p.m.	6.98	34.2	15.0	1.0	60.0
6	Control	11:38 a.m.	7.40	47.2	2.4	1.3	45.1
	Trauma	11:57-12:08 p.m.					
	Post-trauma	1:08 p.m.	7.28	45.5	6.9	3.5	48.3
	Post-trauma	2:08 p.m.	7.22	45.5	7.1	3.6	50.8
	Post-trauma	3:08 p.m.	7.23	43.7	6.5	3.7	52.1
	Post-trauma	4:08 p.m.	7.19	41.8	10.4	3.4	52.5

the effect of hemorrhage and that of muscle trauma upon the plasma phosphorus concentration.

The results of three experiments on hemorrhage and 3 experiments on muscle trauma have been selected to show the distribution of inorganic phosphorus in plasma and in red cells (table 1). It will be seen that the decrease in pH which

follows hemorrhage or muscle trauma is associated with rises in plasma inorganic phosphorus and cell inorganic phosphorus and in most of the experiments an

TABLE 2

*The water and chloride contents of plasma (p) and of cells (c) and the ratio of chloride in cells to chloride in plasma, before (b) and after (s) hemorrhage (H) and/or trauma (T)*

DOG NO.		(H <sub>2</sub> O) <sub>P</sub>	(H <sub>2</sub> O) <sub>c</sub>	Cl(p)	Cl(c)	$\frac{\text{Cl(c)}}{\text{Cl(p)}}$	r <sub>Cl</sub>
		%	%	m.eq./l.	m.eq./l.		
H 1	b	94.7	63.3*	105	64.0	0.61	0.91
	s	95.4	64.3*	108	68.7	0.64	0.95
H 2	b	94.2	63.3*	111	62.8	0.56	0.83
	s	94.7	64.3*	112	69.0	0.62	0.91
H 3	b	92.6	63.3*	100	45.3	0.45	0.66
	s	93.4	64.3*	101	52.6	0.52	0.76
H 4	b	91.2	65.3	98			
	s	91.7	64.8	100	68.9	0.69	0.98
H-T 5	b	92.6	64.8	106	54.5	0.51	0.74
	s	92.3	66.1	109	69.3	0.63	0.89
T 6	b	92.5	62.6	107	53.4	0.50	0.74
	s	92.4	65.1	102	67.1	0.66	0.93
T 7	b	92.6	62.7	114	55.0	0.48	0.72
	s	92.8	63.8	118	63.9	0.54	0.79
T 8	b	93.5	63.4	104	52.2	0.50	0.74
	s	93.4	66.4	104	61.3	0.59	0.83
T 9	b	91.7	63.1	103	57.0	0.55	0.80
	s	91.8	64.1	105	59.8	0.57	0.82
T 10	b	92.7	64.2	113	47.4	0.42	0.61
	s	92.3	63.5	115	72.7	0.63	0.92
T 11	b	93.7	60.1	113	49.5	0.44	0.69
	s	94.0	60.6	113	54.4	0.48	0.74
Aver.	b	92.9	63.3	106.7	54.1	0.50	0.74
	s	93.1	64.3	107.9	64.3	0.60	0.86

\*Average values for cell water assumed.

$$r_{\text{Cl}} = \frac{(\text{Cl})_{\text{c}}}{(\text{Cl})_{\text{p}}} \times \frac{(\text{H}_2\text{O})_{\text{p}}}{(\text{H}_2\text{O})_{\text{c}}}$$

Dog H 3 of this table is dog 2 of table 1.

increase in the organic acid-soluble phosphorus of the red cells. Figure 6 shows that the inorganic phosphorus of the red cells is directly proportional to the plasma inorganic phosphorus. The effect of hemorrhage on the organic acid-

soluble phosphorus of the red cells was studied in 9 dogs. Of these, 6 showed an increase, 1 a slight decrease and in 2 there was no change. Three similar experiments were carried out with muscle trauma. Dogs 5 and 6 (table 1) show small increases after trauma, whereas dog 3 shows a large decrease followed by a return to the control value in the organic acid-soluble phosphorus of the red cells.

Since the changes in the concentration of plasma and cell phosphorus could have been influenced by changes in the water concentration of these compartments, the plasma and cell water were determined. It will be seen in table 2 that no significant changes in the water content of plasma or cells occurred following hemorrhage or muscle trauma.

The chloride concentration of the plasma and cells and the water content of the plasma are of the same order of magnitude as the values reported by Dill et al. (16). On the other hand our cell water values are considerably lower and our  $r_{Cl}$  is much higher than the determinations carried out in the Fatigue Laboratory. The chloride concentration of the plasma is not altered after hemorrhage or muscle trauma, but there is a slight increase in the cell chloride content (table 2), which produces an increase in the ratio of chloride in the cells to chloride in the plasma. This observation agrees with and extends the number of observations of this phenomenon previously studied in this laboratory (3).

**DISCUSSION.** In our experiments reduction of the blood volume by hemorrhage or muscle trauma produces an increase in plasma inorganic phosphorus which is associated with a decrease in blood pH. Between pH 7.4 and 7.1 the plasma phosphorus increases more rapidly after hemorrhage than after muscle trauma. However, when the pH has decreased to 7.0 there is no significant difference in the plasma phosphorus concentration of bled and traumatized dogs.

The increase in plasma inorganic phosphorus concentration of both hemorrhaged and traumatized animals is directly proportional to the increase in the inorganic phosphorus in the red blood cells. Since the concentration of organic acid-soluble phosphorus in the red cells either increases or remains unchanged, the increased inorganic phosphorus cannot come from this source. The fact that marked hyperphosphatemia is observed after hemorrhage as well as after muscle trauma suggests that factors other than mechanical injury of muscle are involved. It is probable that the breakdown of phosphocreatine and other tissue organic phosphates are partially responsible, inasmuch as plasma creatine is said to increase after muscle trauma (12). The work of Guest and Rapoport (15) indicates that acute suppression of renal function is associated with phosphotemia without a reduction in organic acid-soluble phosphorus of the red cells. In this connection we and others (12) have noted an increase in blood NPN following hemorrhage or muscle trauma and it is known that renal function is decreased radically during shock (13, 14).

After hemorrhage or muscle trauma the chloride content of the plasma is unchanged, but the chloride content of the red cells increases. This is probably related to the change in blood pH, for Dill et al. (16) have shown that the chloride ratio of dog's blood increases with decreasing pH.

The blood changes reported in this study can be duplicated in rabbits by inducing gravity shock (17, 18). In this condition the sequence of hemodynamic and

electrolyte changes are similar to those which we have seen in the dog following reduction of the blood volume by hemorrhage or muscle trauma (1, 2, 3).

#### SUMMARY

After hemorrhage or muscle trauma, dog's plasma inorganic phosphorus increases progressively rising abruptly near the time of death. The increase in plasma inorganic phosphorus concentration is directly proportional to the increase in organic phosphorus in the red blood cells. The organic acid-soluble phosphorus of the red cells either increases or remains the same. There are no significant changes in the water content of either plasma or cells after hemorrhage or trauma. The red cell chloride content and the chloride ratio increase, whereas the chloride concentration of the plasma is relatively unchanged.

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# A STUDY OF CERTAIN PROBLEMS RESULTING FROM VAGOTOMY IN DOGS WITH SPECIAL REFERENCE TO EMESIS

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It has been observed in this laboratory that in dogs after double cervical vagotomy frequent vomiting invariably developed (1). This has also been mentioned in other reports (2, 3). However, the mechanism of the development of this disturbance has not been explained nor thoroughly studied.

After a survey of the literature we became more interested in this problem since it is known that the vagus nerves are the important afferent pathways of emetic impulses (4), as evidenced by the fact that stimulation of the vagus nerves may cause emesis (5-7) and that many kinds of induced emesis can be either abolished (4, 8) or greatly ameliorated (9) by vagal sectioning.

It has been invariably reported in the literature that after double vagotomy at a high level in the dog there is paralysis of the esophagus (3, 10-12). The effect of such vagotomy on the cardia seems still to be controversial. Either hypertonia (4) or cardiospasm (13) has been described but contradictory results were also reported (11, 12).

The present experimental work was designed to investigate the mechanism of emesis after vagotomy as well as the nervous control of the esophagus and cardia of the dog.

**METHODS.** Trained adult dogs were used for this investigation. The following studies were made before and after operation. Functional examination of the esophagus and cardia was made on animals lying quietly on the side while a meal containing barium was eaten by the animal or fed through a tube placed in the upper part of the esophagus. Roentgenograms were taken as records whenever necessary. In some trained animals a balloon attached to the end of a catheter was introduced into the upper part of the esophagus and was then inflated. It was allowed to be driven down by the peristaltic waves and the changes of pressure were recorded by a mercury manometer. The esophagus could thus be distended at any desired level and the position checked by aid of the roentgenoscope.

The vagus nerves were severed at different levels in different series of experiments. The animals were always etherized for operative procedures and sterile technic was used. In some animals the vagi were sectioned from the diaphragm upward at different stages. Levels chosen for section were as follows: 1 cm. above the diaphragm, at the hilus of the lungs, at the level of the arch of the aorta, and in the neck. No detailed description of the technic is necessary except that vagotomy in the neck was carried out according to Pavlov's technic; that is, section of the right vagus below the origin of the right recurrent laryngeal branch and section of the left vagus in the middle of the neck. However, this

operation invariably cut the cervical sympathetic fibers. To avoid this an operation was designed to sever all the vagal fibers just above the arch of the aorta. The thorax was entered on the left side through the third intercostal space; all the branches of the left vagus nerve were immediately seized and a short segment was resected between clamps. After a small amount of blunt dissection between the innominate artery and the trachea the right vagus trunk and its branches, usually two to four, could be easily brought out with an aneurysm needle and resected. In this way the esophagus was functionally denervated as much as by the operation in the neck with the advantage that there was no danger of cutting the recurrent laryngeal branch by mistake, and the cervical sympathetic fibers were spared. Observations were made after the animals had recovered from the anesthesia and further studies were made later.

It seemed desirable to observe the acute effect of vagotomy without the complications associated with the operative procedures. Therefore some preparations were made by first cutting all the branches of the right vagus below the recurrent laryngeal nerve and exteriorizing the contents of the left carotid sheath in the neck. In order to facilitate subsequent infiltration of the vagus nerve, the nerve was carefully separated from the other components of the carotid sheath before exteriorization. After complete recovery of the animal from the operation, the vagus nerve could be easily blocked by infiltration of 2 per cent procaine hydrochloride and a temporary condition simulating acute sectioning was thus obtained.

In view of the emphasis put on the rôle played by the sympathetic nervous system in the control of the cardia (14-16) and the manifold sources of sympathetic supply to the cardia (17) and in order to interrupt the sympathetic nerve supply to the cardia and to the esophagus as well, complete sympathectomy was done on a series of dogs, bilateral thoracic sympathectomy on another group of dogs and on a third series bilateral lower thoracic sympathectomy from the sixth costal interspace to the diaphragm.

To some dogs small emetic doses of apomorphine were given subcutaneously before and after full recovery from operation with the intention of seeing if any change in the excitability of the vomiting center took place.

**RESULTS.** *A. The Vagotomized Dogs.* After double cervical vagotomy—that is, the right vagus cut below the origin of the recurrent laryngeal nerve and the left one cut in the neck posterior to the level of the larynx, whether done in two stages at various intervals or in one stage—similar consequences invariably developed. The general impression that dogs become depressed after this operation did not seem to be substantiated. Dogs that were not active before the operation often became less so and sometimes even indifferent to their surroundings, but those originally active often remained as active as before. The heart rate usually increased to 200 or more per minute immediately after vagal section. In the course of a few days it gradually decreased and became more or less fixed at the rate of 140 per minute. This is in accord with previous reports (1, 3). Occasionally the heart rate decreased to only 110 per minute, which was not increased by atropinization but was accelerated to about 130

beats per minute by exercise. The respiration of these animals was generally slow and deep but panting occasionally occurred. The cervical sympathetic nerves were invariably cut when cervical vagotomy was done and bilateral Horner's syndrome was marked in each dog.

Aside from these, the most noticeable disturbance following bilateral cervical vagotomy was the development of frequent emesis, which might occur as soon as the animal recovered from the anesthesia and always remained persistent as long as the animal lived. The dogs raised cotton-like frothy mucus from time to time but in most instances the development of the emesis was associated with feeding. They usually did not show impairment of appetite although they often ate more slowly than before, apparently owing to the accompanying efforts of swallowing. Very often they stopped for a while with the neck stretched and made frequent swallowing and chewing movements. Then they seemed to feel relieved and ate again. The swallowing efforts might become more marked after a certain amount of food was eaten. Suddenly the animal would stand up with legs and neck outstretched and immediately vomit the food just taken. The vomitus formed a sausage-like mass streaked or well mixed with frothy mucus. Sometimes this occurred as a simple effortless regurgitation but frequently there were marked retching movements resulting in active emesis. The dogs then seemed to be greatly relieved and often ate the vomitus and some more fresh food. The whole process was repeated until finally they finished the meal given or became uninterested in the food that remained.

That emesis was not entirely due to the food taken by mouth was evidenced by the following experiments. A number of dogs after double cervical vagotomy were given nothing by mouth. They were given adequate amounts of saline solution with glucose intravenously for various periods. In another series of experiments double cervical vagotomy was performed on dogs with a Mann-Bollman gastric fistula. The animals were fed exclusively through the gastric fistula afterward. In these two series of experiments usually swallowing movements were made occasionally and became very frequent before the onset of vomiting. Often the first part of the vomitus was a copious cotton-like mucus. Sometimes a few retching movements took place with only small amounts of mucus being raised.

In general, in spite of these symptoms, dogs after double cervical vagotomy could live without special care for many months. Usually a few kilograms of body weight were lost gradually in two or three months. Many animals, however, succumbed earlier, the time varying from one to two weeks or longer. At necropsy bronchopneumonia was generally found to be the cause of death but in a few animals there was no apparent cause. The esophagus usually showed various degrees of dilatation at the lower half. The cardiac orifice generally allowed water to flow through freely and admitted one or two finger tips.

When the vagi were sectioned above the arch of the aorta, the dogs showed symptoms not different from those that developed after double cervical vagotomy, except the absence of the bilateral Horner syndrome. Similar, but much

milder, symptoms were observed in dogs after the vagi were sectioned at the level of the hilus of the lungs. After supradiaphragmatic vagotomy the animals did not show difficulty in swallowing and in only a few cases was vomitus occasionally found in the cage.

From these observations it became evident that emesis was inevitable in these animals after high double vagotomy no matter by what route the food was given. Emesis that occasionally occurred after supradiaphragmatic vagotomy was also difficult to understand. It seemed necessary to study the post-operative functional changes in order to find an adequate explanation of the mechanism of emesis.

*B. Functional Examination. a. Roentgenologic studies.* Control studies were made on most animals before operation. The normal esophagus always presented active peristaltic movements. The primary peristalsis was seen after each swallowing movement and seemed not to be different from the secondary waves which started at a lower level, usually in the upper thoracic region above the arch of the aorta, but sometimes at a short distance above the upper border of the sternum. The cardia was seen usually as a short narrow canal which allowed free passage of the bolus of food. There was no spontaneous regurgitation and it was impossible to push the gastric contents back into the esophagus by exerting heavy pressure over the upper part of the abdomen. A soft rubber catheter could be introduced into the stomach practically without resistance. Observations after various operations were made at frequent intervals and will be described in the following paragraphs.

1. *Vagotomy at different levels.* No essential difference in the reactions of the animals from normal could be seen after right or left unilateral cervical vagotomy.

Double cervical vagotomy and section of the vagi above the arch of the aorta produced the same effects. Examination of the animal immediately after recovery from anesthesia or after the incision had fully healed always revealed a paralyzed and more or less dilated esophagus. The barium meal could be seen pushed by the process of deglutition swiftly down to the upper thoracic part of the esophagus. Beyond this level the esophagus was entirely paralyzed and was passively filled until the cardia was reached. When the whole esophagus was filled, a swallowing effort usually made the esophagus more bulging. This was accompanied by the passage of a small amount of barium meal into the stomach through the cardia. As soon as the swallowing wave was over, some of the meal re-entered the upper end of the esophagus and sometimes it would "shoot" into the pharynx. This was followed by swallowing movements and the whole process was repeated. It was observed several times that when the esophagus was distended rapidly there seemed to be a tonic contraction of the esophagus as a whole. As a result, some of the food was pushed into the stomach but a large amount was "shot" into the mouth and vomiting occurred (figs. 1a and b). Examination several hours later often revealed that a part of the barium meal was still present in the esophagus but its amount was generally greatly reduced. Sometimes the barium meal was entirely gone. Apparently

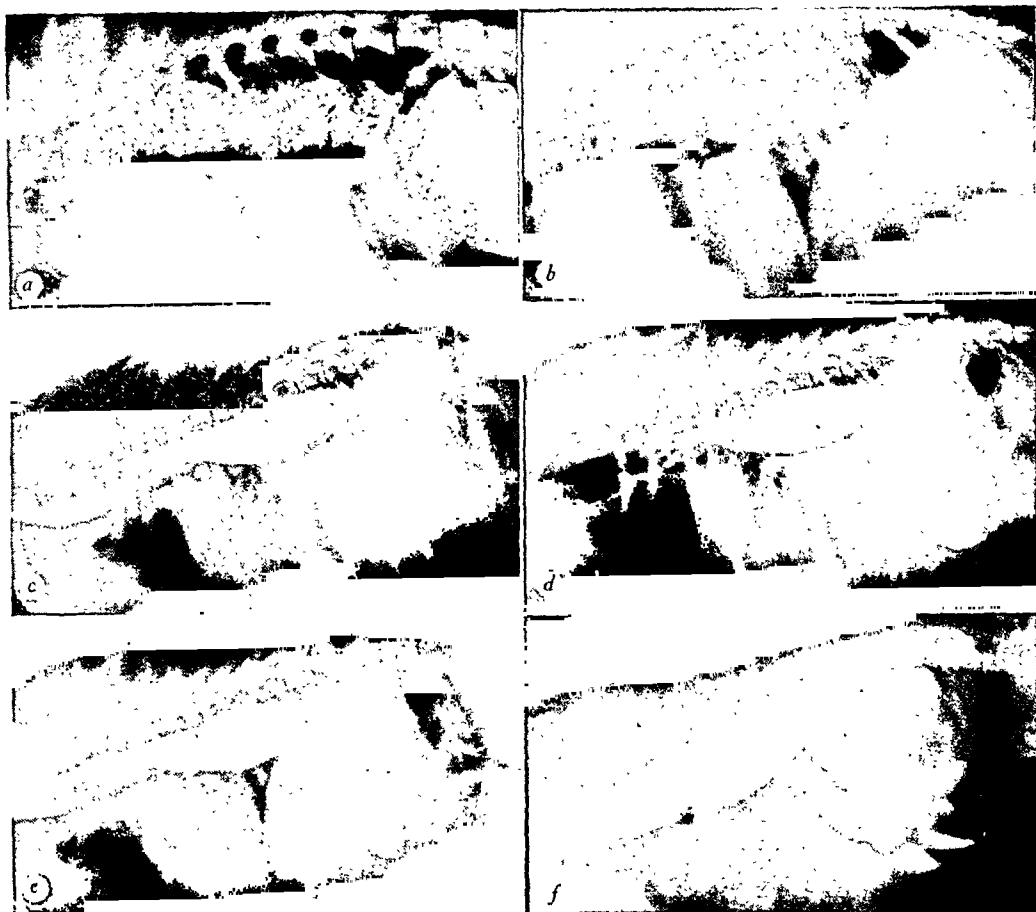


Fig. 1a. The appearance of the esophagus after a barium meal (barium sulfate and meat) is shown in a dog on which double cervical vagotomy has been performed. Note that a considerable portion of the meal has entered the stomach.

b. The same dog shown in a. Frequent regurgitation and swallowing movements were made after the meal. Retching and vomiting occurred about six minutes after the meal. Note that some of the meal is still left in the esophagus while the stomach is nearly empty. The shadow at the right is due to the barium meal given twenty-four hours before, which has reached the colon.

c. Distribution of the barium meal (barium sulfate and milk) in a dog after bilateral cervical vagotomy.

d. Reduction of the amount of the barium meal in the esophagus and an increased amount in the stomach after the animal (same as shown in c) had been held upright for five minutes. A repetition of the same procedure did not further reduce the amount in the esophagus.

e. The appearance of the esophagus and stomach of the same dog as in c and d after manual pressure had been applied over the upper part of the abdomen. The barium meal in the stomach was pushed into the esophagus. Note the increase of the amount of the barium meal in the esophagus and reduction in the stomach as compared with d. A small amount of the barium meal has entered the small intestine.

f. Condition of the cardia of a dog after supradiaphragmatic vagotomy. All of the barium-milk mixture entered the stomach. Manual pressure over the upper part of the abdomen forced the gastric contents back into the esophagus as shown here but the barium will be driven into the stomach again. Spontaneous regurgitation may follow as described in the text.

it was regurgitated or vomited or it was forced into the stomach as a result of the accumulation of salivary and esophageal secretions aided by the swallowing movements.

During periods of observation of ten to thirty minutes with the animal lying quietly on its side, the barium meal retained in the esophagus usually remained unchanged in amount. After the animal had been held in an upright position for five minutes or so, there was always some reduction of the amount of the meal in the esophagus and at the same time the stomach contents were increased (figs. 1c and d). Repeated similar procedures did not produce further change. This might imply that the tone of the cardia was sufficient to resist a certain amount of hydrostatic pressure. However, when the soft rubber catheter was introduced, it passed through the cardia easily without giving a sensation of increased resistance. In addition it was possible by manual pressure over the upper part of the abdomen in some animals to push back the gastric contents into the esophagus (fig. 1c). From the findings just described the deduction may be made that in these animals the tone of the cardia, although definitely decreased in some cases, was still sufficient to resist a certain amount of pressure. However, the paralyzed state of the esophagus and negative intrathoracic pressure should be considered as possible factors in the retention of food in the esophagus when the animal was held in an upright position.

Eighteen dogs were used for all of the foregoing experiments. Only ten of them were tested by exerting pressure over the upper part of the abdomen. The condition of hypotonia of the cardia was found in five dogs. In two of them the vagi had been sectioned just above the arch of the aorta; in the other three double cervical vagotomy had been performed.

Dogs with vagi sectioned at the level of the hilus of the lungs showed paralysis of the lower fourth of the esophagus. There was no difficulty for the meal to enter the stomach but the last portion of the bolus of food was retained in the esophagus. The peristaltic activity of the normal part of the esophagus appeared to push the bolus into the paralyzed part, which was rapidly distended. Up to a certain point the cardia was open and the meal entered the stomach. As soon as the peristaltic contraction was ended, the contents of the distended lower fourth of the esophagus were thrown back to the upper part of the esophagus. The process was similar to the condition produced by section of the vagi at a higher level as already described, except that a much smaller part of the esophagus was involved. Tests by means of the soft rubber catheter did not reveal resistance at the cardia. It was possible to push the contents of the stomach into the esophagus by manual pressure in each of the three dogs examined.

Findings in dogs after supradiaphragmatic vagotomy were consistent. The esophagus showed normal activity in driving the bolus of food into the stomach. However, as soon as the peristaltic wave was ended or after a brief interval the contents of the stomach were rapidly regurgitated into the esophagus, rushing up often to the upper thoracic part and sometimes to the cervical region. In most cases secondary waves of the esophagus were induced and the regurgitated

meal was once more driven down into the stomach. The same process was then repeated but each time the amount regurgitated became less and less. Finally the entire meal entered and remained in the stomach. The amount regurgitated, the height reached by the food in the esophagus and the number of regurgitations before the complete clearance of the esophagus were closely related to one another in a given animal but varied in different animals. After the meal had completely entered the stomach, it was possible in four of the nine animals studied in this series to push the stomach contents back into the esophagus by exerting manual pressure over the upper part of the abdomen (fig. 1f). Vomitus was occasionally found in the cages of those animals that showed the most marked regurgitation.

The findings just described could be readily explained by the atonic condition of the cardia and the impairment of the receptive relaxation of the stomach. The receptive relaxation of the stomach was demonstrated in cats and one human subject by Cannon and Lieb (18), who showed that in cats it was controlled reflexly by the vagus nerves. They in fact predicted that "if intragastric pressure remains high and the cardia were patulous, the contents of the stomach might be pressed in large volume up to the esophagus after each act of deglutition." This seemed to be what happened in these dogs after supradiaphragmatic vagotomy. In contrast to this view was the report that the receptive relaxation of the stomach was still present in unanesthetized dogs after the vagi had been sectioned above the diaphragm as studied by the balloon method (12, 17). However, the receptive relaxation of the stomach after vagotomy might be less than normal. In such a case the relaxation might not be sufficient to accommodate a large amount of food and regurgitation would occur if the cardia were atonic. We never observed a similar regurgitation in dogs with vagi sectioned at a higher level, probably because the amount of food discharged into the stomach from the paralyzed esophagus was much smaller.

The persistent finding in this series of experiments that the cardia was in an atonic condition needs some consideration. In the first place, since it seems to be generally believed that the sympathetic supply to the cardia is motor in function (13, 14, 15, 19) and since it has been reported that the vagus nerves are joined by sympathetic fibers below the hilus of the lungs where they form a plexus over the esophagus (16), it must be recognized that section of the vagi above the diaphragm would have cut such sympathetic fibers at the same time. This possibility seems to have been ruled out by the finding that in dogs the cardia of which had been freed of sympathetic influence from this source by bilateral thoracic sympathectomy there was found neither spontaneous regurgitation nor a patulous cardia. In the second place, it seems to be generally accepted that the vagus nerves contain both motor and inhibitory fibers to the cardia. This has been repeatedly reported by numerous investigators working with different methods on various species of animal, chiefly rabbits (19-22), cats (18, 19, 23) and dogs (19, 20). Sinnhuber (24) in 1903 reported that vagotomy above the diaphragm in dogs resulted in an atonic cardia while vagotomy in the neck caused temporary hypertonicity. In regard to this Carlson and

Luckhardt (25) expressed their opinion that "if these observations should prove to be correct, it would seem that in the dog the inhibitory fibers to the cardia leave the vagi at some distance above the diaphragm and pass down to the cardia in the wall of the esophagus." Recent experiments made on dogs under barbital anesthesia with a modified Langley esophageal manometer gave results in strong support of this view (13); that is, the motor fibers of the vagus to the cardia appeared to be present in the vagus trunks while the inhibitory fibers seemed to run an intrinsic course in the esophageal wall. Studies made on unanesthetized dogs also indicated such an intrinsic course for the inhibitory fibers to the cardia. These studies suggested that these fibers were given off by the vagi above the hilus of the lungs since the inhibitory response of the cardia was still present after section of the vagi at this level but was abolished after section of the vagi in the neck, although no hypertonicity of the cardia was found (12).

The evidence obtained from the present experiments on dogs on which supradiaphragmatic vagotomy had been performed and which were studied under highly physiologic conditions seemed to give support to this concept. This might be expected, since section of the motor paths would leave the inhibitory mechanism free to express itself. However, in the experiments of the first series with the vagi sectioned above the arch of the aorta or higher, although none manifested hypertonicity of the cardia, there were definite individual differences. A patulous cardia was shown in some dogs but none in the others. These inconsistent results do not solve the problem of the level at which the inhibitory fibers are given off. It seemed to be indicated that vagal section at different levels in the same animal should be carried out.

Some experiments in which this was done will now be considered. Two of the dogs showing patulous cardia after supradiaphragmatic vagotomy were chosen for study. In one dog the operations for sectioning the vagi at the hilus of the lungs and above the arch of the aorta were done in successive stages. After each stage of operation, the cardia was found as patulous as before. Therefore, another double vagotomy was done in the neck with the right vagus sectioned below the recurrent laryngeal nerve. Examination on the second day showed that the barium meal could still be pushed back into the esophagus but a stronger pressure was required. On the third day, while the animal was under local anesthesia, the right vagus was sectioned in the neck just below the level of the larynx. Immediate examination and examinations made on the second and the fifth day showed the cardia to be in the same condition as before this operation. This animal died on the tenth day without apparent cause.

Double cervical vagotomy was performed on the other animal three months after the supradiaphragmatic vagotomy. There was no apparent change in the tone of the cardia immediately following, nor as long as three months after, the operation, since the barium meal in the stomach could still be pushed back into the esophagus.

A third dog that showed a patulous cardia after section of the vagi just above the arch of the aorta was again given double cervical vagotomy three weeks later. The cardia still allowed the barium meal in the stomach to pass through

when strong pressure was applied to the abdomen. One month later the right vagus, which had been sectioned posterior to the recurrent laryngeal nerve, was now severed in the middle of the neck. After this operation the cardia was found to have a tonus comparable to that of a normal animal. In this animal it was observed that the peristalsis of the upper third of the esophagus was still present, although the vocal cords were fixed in the cadaveric position. When this paper was written, this animal was still alive three months after both vagi had been sectioned just posterior to the larynx.

The experiments just described would suggest that the vagal supply of the cardia is chiefly motor in function. Some fibers probably leave the main nerve trunk at a high level, in all probability between the arch of the aorta and the base of the neck, where they take an intrinsic course in the esophageal wall and exert mainly an inhibitory action on the cardia.

2. *Sympathectomy.* This series of experiments was done in order to see the effects produced by sympathetic denervation of the esophagus and cardia. Bilateral thoracic sympathectomy was usually carried out in two stages, which were followed by complete abdominal sympathetic ganglionectomy in a single stage. Observations were made on three such animals.

The findings after bilateral thoracic sympathectomy were practically the same as before the operation. No change in the activity of the esophagus was observed. Spontaneous regurgitation of food through the cardia was not seen and the barium meal in the stomach could not be pushed back into the esophagus. After the abdominal sympathectomy the same finding was obtained except in one dog. In this animal it was possible to push the contents of the stomach through the cardia into the esophagus but fairly strong pressure was required. In general all these animals behaved just like the normal ones. All the examinations with the barium meal in this series were made at least two weeks after the operation and were repeated several times at intervals.

Knight (15) reported increased esophageal activity and patulous cardia after bilateral thoracic sympathectomy (third to tenth) and marked hypertonicity in the upper third (striped muscle) of the esophagus after bilateral excision of the stellate ganglia in cats examined after a barium meal given while the animals were under light ether anesthesia. Our results after bilateral thoracic sympathectomy (first to twelfth) were not in accord with the findings of Knight, perhaps owing to differences both in the species of animal studied and in the experimental conditions under which the observations were made. Excision of the splanchnic nerves did not influence the cardia in the cat (2) or in the dog (12), but a permanent patulous cardia developed in cats after celiac sympathectomy (15). This latter finding was later applied to human cases of achalasia of the cardia but the results were not encouraging. After careful dissection of fourteen infants, Mitchell (16) ascribed the clinical failure to the incomplete interruption of the sympathetic supply to the cardia, since the cardia received sympathetic fibers from multiple sources. In the present series of completely sympathectomized dogs only one of the three showed moderate decrease of the tone of the cardia while the other two showed apparently normal function. This incon-

sistency of the results prevents any final conclusions as to the effect of sympathectomy on the cardia. It is definitely demonstrated, however, that even complete sympathectomy will not always give rise to an atonic cardia.

3. *Vagotomy combined with sympathectomy.* After various periods of observation, double cervical vagotomy was done on the three sympathectomized dogs just described. Unfortunately two of them died on the third day after operation and the other one died on the second day. Pneumonia was the cause of death in each case. However, all three animals showed typical symptoms and roentgenologic appearance of the esophagus following vagotomy. When the animal was held in an upright position for five minutes or longer a certain amount of the barium meal was still retained in the paralyzed esophagus. By exerting pressure over the upper part of the abdomen it was revealed that the tone of the cardia was decreased in one of the two dogs the cardia of which remained apparently normal after sympathectomy. The other two showed no change of their cardia after the vagotomy; that is, the contents of the stomach could be pushed back in one but not in the other. These observations were made at least two to four hours after the animals had recovered from the ether anesthesia. In view of the fact that in all the previous studies on the effect of vagotomy on the cardia the roentgenologic findings obtained shortly after recovery from the anesthesia usually persisted, the effects of cervical vagotomy of the sympathectomized dog may also be persistent.

In two dogs bilateral lower thoracic sympathectomy was done combined with supradiaphragmatic vagotomy. The sympathectomy included the sympathetic chain from the sixth to the twelfth thoracic vertebra and the splanchnic nerves were definitely cut on both sides. The idea is that if the sympathetic nerves to the cardia are chiefly motor in function their ablation would leave a very patulous cardia, since it appeared that the vagus trunks are also motor to the cardia. As a result of the combined operation, the contents of the stomach could be pushed back into the esophagus but a considerable amount of force was required. In one case spontaneous regurgitation occurred but this took place a short time after the meal got into the stomach, not so rapidly as in the case of vagotomy alone. In the other animal there was no spontaneous regurgitation at all.

b. *Balloon examination.* A balloon attached to the tip of a rubber catheter was used in trained dogs before and after various operations to explore the condition of the esophagus and cardia. The mouth of the dog was kept open while the dog was lying quietly on its side. The balloon was gently introduced into the upper part of the esophagus and was then inflated with various amounts of air. In normal dogs an inflation with 20 cc. of air was usually the threshold stimulus to excite the esophageal peristaltic contraction. The period of latency seemed to vary with different amounts of inflation. Usually 40 or 50 cc. of air injected into the balloon served the purpose satisfactorily. The inflated balloon was allowed to go freely down the esophagus but it stopped as soon as the cardia was passed. Along its course a typical curve was always registered (fig. 2a). The first peak after the initial rise of pressure corresponded to the beginning of the esophageal contraction while the second was apparently due to the passage of the

balloon through the cardia into the stomach. After the balloon entered the stomach further changes of pressure were not observed.

The typical finding after the vagi were sectioned at different levels was that the balloon at first rapidly descended. This was shown on the kymographic tracing as the first peak of the normal curve. After a certain time the balloon stopped and remained in the lower or paralyzed part of the esophagus, after which the tracing showed fluctuations corresponding to the respiratory move-

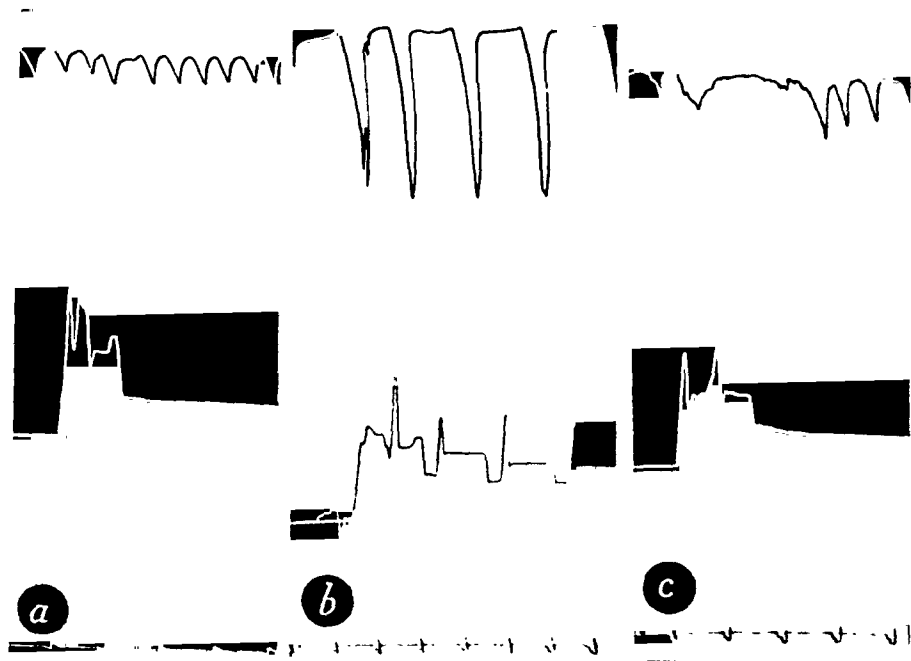


Fig. 2a. A typical kymographic tracing in a normal dog made by the balloon method in the examination of the esophagus as described in the text. Forty cubic centimeters of air had been injected as indicated by the initial rise. The following peak represents the beginning of the esophageal peristalsis. The last peak marked the passage through the cardia. Pressure changes were not shown after the balloon got into the stomach.

b. A typical tracing by the same method in a dog after cervical vagotomy. The balloon was inflated in the upper part of the esophagus and was carried on by the peristaltic wave. However, it stayed in the paralyzed esophagus and showed pressure changes corresponding to respiratory movements.

c. A typical tracing made by the balloon method of examination in a dog after supra-diaphragmatic vagotomy. The balloon was driven down into the stomach but the last peak of the curve was absent in this case.

ments (fig. 2b). The animal rested quietly while the balloon remained in the paralyzed part of the esophagus for various periods. In dogs after supradia-phragmatic vagotomy, the balloon passed into the stomach easily, but the second peak of the tracing was very small or absent, especially when the cardia was particularly loose (fig. 2c). A tracing not different from normal was still obtained in dogs after complete sympathectomy or bilateral thoracic sympathectomy. All these findings are quite in accord with the observations made by roentgenologic means.

If the inflated balloon was held stationary in the esophagus of a normal dog by means of the rubber catheter, there was experienced a considerable pull. With an inflation of 50 cc. or less of air, the balloon was modeled into a long slender sac, a part of which might be pushed into the stomach periodically when it was placed in the lower part of the esophagus. A silk bag was used to cover the balloon and a strong thread was fixed to the bag inside the lumen of the catheter in order to make the balloon and the bag nonstretchable. In this way the power of the peristaltic drive of the esophagus against the retained balloon was recorded

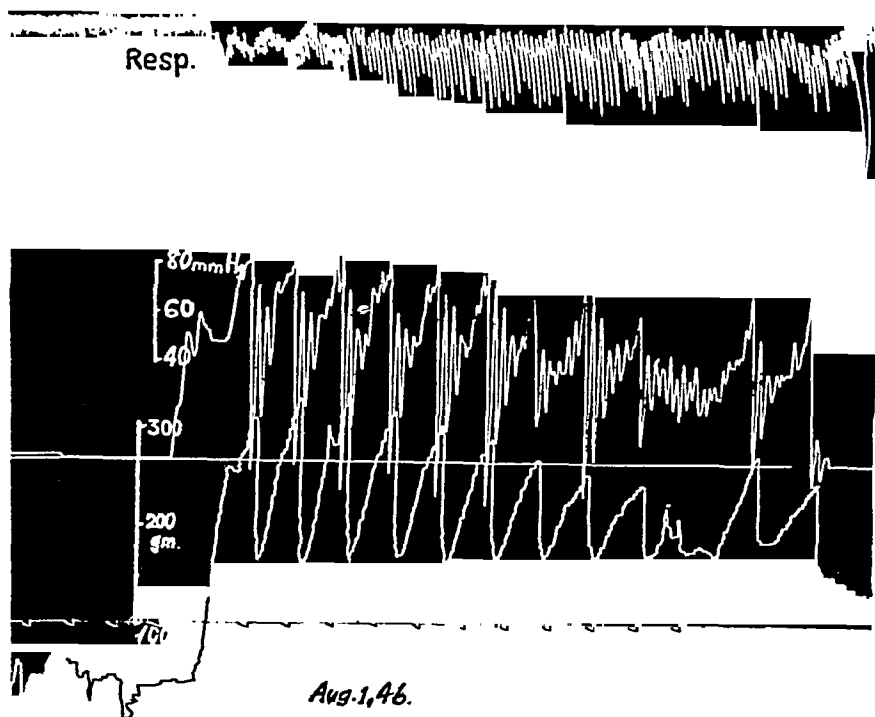


Fig. 3. The effect of retention of a balloon in the esophagus of a normal dog. Both the tube and the balloon were made nonstretchable and the balloon was inflated with 30 cc. of air and placed in the middle of the esophagus. Reading from above downward: respiration, pressure changes in the balloon as recorded by a mercury manometer, force of the esophageal drive against a spring balance and time in five second intervals. Notice the rhythmic secondary peristaltic waves superimposed on a tonic contraction of the esophagus.

by use of a spring balance and the pressure changes inside the balloon were registered at the same time by a mercury manometer (fig. 3). The inflation of the balloon was soon followed by secondary peristaltic contractions of the esophagus at a rate of from four to fourteen each minute. The maximal force of the pull of the esophagus varied from 240 to 400 grams. This is in accord with a previous report (26). Only the respiration of the animal was affected. It should be pointed out here that there was a tonic contraction of the esophagus amounting to 180 grams. This tonic contraction usually lasted from fifteen

seconds to one and a half minutes and was closely associated with these secondary peristaltic waves.

When an ordinary balloon was placed in the middle of the paralyzed esophagus and gradually inflated, the animal seemed not to be affected at the beginning (fig. 4). However, when the balloon was inflated to a volume from 150 to 200 cc., corresponding to a pressure varying from 40 to 60 mm. of mercury, the animal would show chiefly some respiratory changes and the esophagus seemed to contract as a whole. As a result, the balloon entered into the stomach. The same result was obtained in dogs immediately and several weeks after double vagotomy at any high level. When the vagi were cut at the hilus of the lungs, distention with 100 cc. was usually sufficient to excite the reaction. A satisfactory examination was not obtained in dogs on which complete sympathectomy and double cervical vagotomy had been performed. However, in no case did nausea or vomiting develop on such distention. Both in the normal and in the vagotomized dogs, if the balloon was retained in the upper third of the esoph-

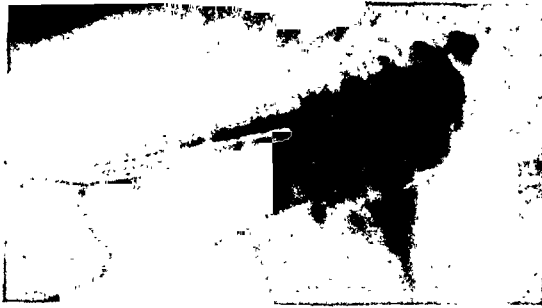


Fig. 4. Extreme effect of double cervical vagotomy. The balloon was inflated in the paralyzed esophagus. When 300 cc. of air were given, the animal showed no tendency to vomit. On further inflation the animal showed discomfort and the balloon was pushed down into the stomach.

agus and distended with air there was often vomiting. But on distention of the balloon at that level pharyngeal irritation was not avoided.

c. *The immediate effect of vagal block.* Two dogs with the right vagus nerve sectioned below the origin of the recurrent laryngeal nerve and the left vagus exteriorized in the neck showed no essential difference from the normal dog. Studies were made after infiltrating the exteriorized left vagus nerve with 2 per cent solution of procaine hydrochloride. Within three minutes after the injection of procaine the heart rate was rapidly accelerated and Horner's syndrome fully developed in two or three minutes. At the same time observations were made of a barium meal which failed to go through the cardia. The esophagus showed a rapidly ascending paralysis as far as the upper thoracic portion. If the animal was given food, it did not show impairment of appetite but repeated regurgitation and vomiting occurred. Examination by means of a balloon after vagal block was also made. In every respect the results following infiltration of the vagus were not different from those obtained in dogs following double cervical vagotomy. Recovery from the procaine block occurred within thirty to

forty minutes. The esophageal contraction rapidly became effective, reached the lower segment of the organ and carried all the retained food into the stomach and the animal showed no more symptoms. The Horner syndrome of the left side rapidly disappeared at the same time. The heart rate was the last to recover, taking more than one hour.

Animals with one vagus sectioned and one exteriorized could be kept in perfect condition indefinitely. Vagal block could be produced at any time and also it could be maintained for any desired period by repeated injections of procaine.

d. *The pharyngeal reflex.* A number of the trained dogs showed an increased response to pharyngeal stimulation after vagotomy at the level of the hilus of the lungs or higher. This was not noticed until the balloon examinations were being made. After vagotomy, touching the balloon to the pharynx often excited nausea and sometimes vomiting, which never occurred under the same experimental conditions before the operation. This condition was only slightly evident in some dogs but very marked in others. In a few dogs it was marked in the first week and became milder but persistent afterward. In general the balloon seemed to be much more irritating than a rubber catheter. In many cases an exaggerated reflex was not noticed when the rubber catheter was used to introduce the barium meal but introduction of a balloon was impossible. While some animals were sensitive to the balloon as well as the tube, many cases may have been missed since the balloon examination was not made on all animals. However, none of the dogs examined showed this phenomenon after supradiaphragmatic vagotomy.

When the vagi were sectioned at a higher level the pharyngeal reflex was exaggerated in eight of thirteen dogs. One of these dogs did not show this exaggeration of the reflex when the vagi were cut just above the diaphragm but a definitely increased pharyngeal sensitivity was present after vagal section at the hilus of the lungs. There seemed no further increase of the sensitiveness of this region when the vagi were subsequently cut in the same dog at higher levels. Another two dogs with vagi sectioned above the diaphragm also showed the same phenomenon after double cervical vagotomy. One of the two dogs with exteriorized vagus showed a marked increase of this reflex after the vagal block. However, in many of these mild cases balloon examination was still possible when proper care was taken. As soon as the balloon was gently pushed through the pharynx, it could be placed in the esophagus without further reaction on the part of the animal.

e. *The response to apomorphine.* In a series of dogs small effective doses of apomorphine were given before and after recovery from the various operations. Fresh saline solution of the drug was used each time. The animals were always fasted for eighteen hours before the apomorphine was given subcutaneously at the nape. The animals were allowed their freedom in the laboratory and no repetition of the test was made on the same day. Under these conditions a preliminary study revealed the response of each dog to the same dose of the drug to be fairly consistent. In general it could be seen that the response was exaggerated after double vagotomy (table 1).

TABLE 1  
*Effect of apomorphine given subcutaneously to dogs*

ANIMAL	CONTROL TEST					AFTER OPERATION				
	Condition	Apo- mor- phine	Emesis	Inter- val before emesis	Times re- peated	Operation	Apo- mor- phine	Emesis	Inter- val before emesis	Times re- peated
		mgm. per kgm.		min.			mgm. per kgm.		min.	
1	Right vagus cut below recurrent laryngeal	0.036 0.036	+ +	7 6	2 1	Left vagus cut in neck	0.036	+	3	3
2	Right vagus cut below recurrent laryngeal	0.055 0.04	+ +	3 6	6 1	Left vagus cut in neck	0.04 0.04	+ +	3 2	3 7
3	Right vagus cut below recurrent laryngeal and left vagus ex- teriorized in neck	0.048 0.024 0.024	+ + +	3 4½ 6	5 3 1	Left vagus block- ed with pro- caine hydro- chloride	0.024	+	10	5
4	Normal	0.025	+	7½	0	Supradiaphrag- matic vago- tomy	0.025	+	10	1
5	Normal	0.04 0.04 0.04	+ + +	4 5 5	1 4 1	Supradiaphrag- matic vago- tomy	0.036 0.036 0.036	+ + +	5 4 5	7 2 3
6	Normal	0.038 0.038	+ +	5 4	0 1	Supradiaphrag- matic vago- tomy	0.038 0.038	+ +	5 6½	2 2
						Double cervical vagotomy, one stage	0.035	+	3	6
7	Normal	0.03	+	5	2	Vagi cut at the hilus of the lungs	0.03	+	4	3
8	Normal	0.03	+	4	0	Thoracic sympa- thectomy	0.03 0.03	+ +	3½ 13	0 0

The average effective dose of apomorphine in this series of experiments was about 0.03 mgm. per kilogram, which is much smaller than that reported by Eggleston and Hatcher (27). They found the minimal dose by subcutaneous injection to be about 0.1 mgm. per kilogram and the average effective dose was

0.2 mgm. per kilogram. The probable explanation for these results is a difference in potency of the drug. (The drug used in this series of experiments was the apomorphine hydrochloride tablets made by Sharp and Dohme Company.) But probably the mental state of the animal should be noted, as illustrated by the following experiment.

One normal dog received 0.03 mgm. for each kilogram of body weight subcutaneously and was allowed the run of the laboratory. Four minutes later it vomited three times. Next day the animal was restrained on a table in a semi-dorsal position after the manner of Eggleston and Hatcher (27). The same dose was given but neither vomiting nor the usual symptoms of licking were observed in one hour. The dog was not accustomed to this position and struggled from time to time. On the following day the same experiment was repeated. This time the animal seemed to be accustomed and was lying quietly in the semi-dorsal position. Five minutes after administration of the same dose of apomorphine the dog vomited twice.

Another dog was given apomorphine once every morning with a few exceptions. Altogether seven injections were given in ten days. Vomiting was obtained each time and the results were quite uniform. When the same amount of saline solution was given at about the usual site of injection of apomorphine no response was ever observed. This would indicate that a conditioned reflex was not established in this short period and the results of repeated doses of the drug are not explained on such a basis.

COMMENT. After a careful examination of the dogs before and after vagotomy at a high level, the main disturbance seems to be a paralysis of a certain portion of the esophagus, which is passively distended with food during a meal, part of the food being retained in the esophagus. But these two factors, as pointed out before, do not seem to account for the development of the emesis. However, as a result of distention either with food or with a balloon as observed by aid of the roentgenoscope, the paralyzed portion of the esophagus often responds with a generalized tonic contraction. In the normal dog this tonic contraction of the esophagus is very apparent (fig. 3). This has also been noticed by Carlson and Luckhardt (28). Further work seems necessary to tell whether it is due to the independent nature of the muscle of the esophageal wall or to stimulation of some intrinsic nervous mechanism but the evidence presented in this report indicates that the extrinsic vagal supply is not concerned.

This property of tonic contraction of the vagotomized esophagus apparently is important and aids in forcing the food down to the stomach during swallowing. On the other hand it seems to be also responsible for the regurgitation of food into the mouth. The accumulated food in the paralyzed esophagus did not seem to pass readily through the comparatively narrow cardia. In some cases the cardia was loose enough to permit food to be pushed from the stomach into the esophagus by manual pressure. Apparently this manual pressure was stronger than the tonic contraction of the paralyzed esophagus plus the push exerted by the act of deglutition. Probably on account of this the food tended to be regurgitated as soon as the swallowing wave was ended. Why the balloon always entered the stomach instead of being regurgitated could not be readily

understood. Probably the presence of the tube in the upper part of the esophagus helped to prevent it from being expelled and also there might be some mechanical dilator effect at the cardia.

The emesis in most dogs occurred as a result of repeated regurgitation. It seems probable that irritation of the pharynx with large amounts of regurgitated food is responsible for the development of the active emesis. In support of this concept, it was found in many dogs that the response to pharyngeal stimulation was more sensitive than normal and the response to the administration of apomorphine was exaggerated. The latter might mean a more sensitive vomiting center and might be the explanation of the former but we do not have sufficient data to test this possibility in the same group of dogs. The same factors are probably concerned in the development of emesis in vagotomized dogs when nothing is given by mouth. These dogs usually have large amounts of secretion accumulated in the paralyzed esophagus. Efforts were made in these dogs to abolish the pharyngeal reflex with cocaine. However, we failed to obtain a satisfactory proof of our hypothesis in this way because two of these dogs with cocaine-pharynges were almost choked to death soon after they started eating.

This kind of emesis does not seem to require the integrity of the sympathetic nervous system. This is suggested by the fact that in the completely sympathectomized dogs similar phenomena developed after double cervical vagotomy. The development of the emesis, the exaggerated response to pharyngeal stimulation and the response to apomorphine could not be explained by irritation of the cut ends of the sectioned nerves after the operation in the early stage nor on the basis of the neuroma formed later, since all these phenomena were obtained in the dog after vagal block.

Hatcher and Weiss have expressed the belief that the action of apomorphine is to increase the excitability of the vomiting center so that the normal afferent impulses from different parts of the body induce vomiting (4). But in their original article (29) they suggested that apomorphine induces emesis by rendering the center hyperexcitable to normal afferent impulses that pass by way of the "sympathetic type of nerves" from many organs, as evidenced by the failure of apomorphine to cause emesis in two dogs after administration of ergotoxine phosphate which they stated paralyzes sympathetic nerve endings. One of our completely sympathectomized dogs received apomorphine (0.05 mgm. per kgm.) and vomited twice. Therefore the impulses from vagus nerves ("sympathetic type" as Hatcher and Weiss (29) suggested) and other sources still seem to play an important rôle. After section or block of the vagus nerves, which are the important paths of afferent emetic impulses, not only did frequent vomiting develop, but the response to the apomorphine was also exaggerated. On the basis of the small amount of data available, we do not like to venture the suggestion that some normal inhibitory effect on the vomiting center is removed after vagotomy, but this possibility should be borne in mind.

The classic conception appears to be well founded that the co-ordinated peristaltic movement of the esophagus depends on the integrity of the extrinsic vagal supply. But the conception that the upper part of the esophagus is supplied by the recurrent laryngeal nerve seems to need reconsideration, for we have ob-

served in one dog that the peristaltic activity of the upper third of the esophagus was not at all impaired even after both recurrent laryngeal nerves had been interrupted. Work is now in progress to investigate this point.

Jurica (2) reported the recovery of some activity of the striated muscle of the upper part of the esophagus in cats within three months or earlier after bilateral vagotomy in the neck. On two occasions we observed a similar phenomenon in the lower third of the esophagus in dogs after vagotomy. A series of shallow waves of constriction were seen running one after another from the level of the diaphragm up to the middle of the thorax. This spontaneous activity did not move the barium meal in the esophagus and the dog remained quiet. This phenomenon was observed twenty-nine days after double cervical vagotomy in one dog, and twenty-four days after vagal section above the arch of the aorta and the third day after double cervical vagotomy in the second dog. Later examinations did not reveal this kind of motility. Since this phenomenon was not observed constantly and was not associated with any symptoms or physiologic effect, it may not be of important functional significance.

The results obtained in our experiments with a barium meal in trained dogs give support to the view held by recent workers (12, 13) as to the manner in which the cardia is innervated by the vagus. From these studies the suggestion is offered that the intrinsic inhibitory fibers to the cardia are probably given off at a level above the arch of the aorta. However, the presence of inhibitory fibers in the vagus trunks also seems possible, since the receptive relaxation of the cardia is reported not to be abolished by an encircling incision around the lower part of the esophagus (30). On the whole, we considered the vagal supply to the cardia to be mainly motor in function since in no case could we demonstrate hypertonia of the cardia after double cervical vagotomy while hypotonia was found in many of these cases. This is in accord with previous reports (11, 12). However, the symptoms of dysphagia and regurgitation, and the roentgenologic findings in these dogs are very much like the typical clinical cases of cardiospasm or achalasia of the cardia. Of course we do know that these symptoms in these dogs are primarily a result of the esophageal paralysis. The food is retained in the esophagus on account of the failure of the esophagus rather than obstruction at the cardia, which is in many cases even hypotonic. Because of the difference of species and especially the difference in the structure of the esophageal musculature it seems impossible to talk about the analogy of the condition in these vagotomized dogs with human cases of cardiospasm. But it is really surprising to find how closely the findings in a recent study of the esophagus of human cases of cardiospasm (31) approach those reported in the present communication.

#### SUMMARY AND CONCLUSIONS

1. An experimental study has been made in the dog before and after double vagotomy at different levels from the diaphragm anteriorly to the larynx. Particular attention has been paid to the mechanism of emesis after vagotomy at high levels as well as the nervous control of the esophagus and the cardia.

2. The functional changes after the various operations were studied roentgenologically with the aid of barium meals and catheterization with a soft rubber tube. Balloon studies were also made in many experiments.

3. The general behavior of these animals after the various operations has been described in detail. The development of frequent regurgitation and emesis was a common consequence after vagotomy was done at, and especially higher than, the level of the hilus of the lung. Similar, although much less severe, phenomena occurred when nutrition was maintained only by parenteral feeding.

4. The most important change after vagotomy at a high level in the dog is a complete loss of the peristaltic activity of the lower part of the esophagus. The lower two thirds of the esophagus were involved when vagotomy was done at any level above the arch of the aorta.

5. The paralyzed esophagus was shown to retain food material or secretions which might be either partly pushed down to the stomach or partly regurgitated, apparently by the aid of the diffuse tonic contraction of the whole esophagus as a response to the rapid distention. Mere retention of the food in the paralyzed esophagus or distention of the esophagus was not shown to be the cause of the nausea and vomiting.

6. The nausea and vomiting in these animals apparently resulted from irritation of the pharynx by the large amount of the regurgitated material. The response to the pharyngeal irritation was found to be more sensitive in many dogs after than before vagotomy. Coincidentally in another series of experiments the response to appropriate doses of apomorphine was found to be greater after than before vagotomy, a result that suggests a hyperexcitable status of the vomiting center.

7. The possibility of irritation of the sectioned nerve stumps as the cause of vomiting was eliminated by similar findings in dogs after the exteriorized vagus nerve had been blocked with procaine hydrochloride.

8. Similar consequences occurred after vagotomy in dogs on which complete sympathetic ganglionectomy had been performed.

9. After complete sympathetic ganglionectomy two dogs did not show any significant change of the function of the esophagus and the cardia and in a third dog the tone of the cardia was only slightly reduced.

10. The peristalsis of the lower two thirds of the esophagus is dependent on the extrinsic vagal supply. But it is not likely that the nerve supply to the upper third is derived from the branches arising from the vagus posterior to the larynx.

11. The vagus contains both inhibitory and motor fibers to the cardia. There is evidence that the inhibitory fibers branch off from the main vagus trunks above the level of the arch of the aorta and take an intrinsic course in the esophageal wall to reach the cardia.

12. When the cardia was freed from vagal control its tone was never found increased but was reduced to different degrees in the majority of cases. In one dog the tone of the cardia was found not changed after complete sympathetic ganglionectomy but was reduced by subsequent double cervical vagotomy.

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# BODY TEMPERATURE AND OXYGEN CONSUMPTION IN TRAUMATIC SHOCK AND HEMORRHAGE IN MICE

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Previous investigation has established that severe trauma will bring about a fall in body temperature (1) (2) (15) and in metabolic rate (3) (4) (2). These changes have been attributed to circulatory impairment, to anoxia present in shock, and to increased heat loss.

With the development of standardized procedures for the production of shock, and of certain therapeutic measures that can bring about survival, it was of interest to investigate temperature and oxygen consumption under these conditions, with particular reference to the effects of therapy.

All experiments were done upon mice. Their relatively large surface area makes them quite susceptible to environmental temperature (5). The magnitude of the changes in body temperature offers certain advantages for the purpose of this investigation. In the application of these results to larger animals it must be remembered that body temperature fluctuations secondary to a changing environment or to metabolic disturbances may not be as great.

**METHODS.** Burn and tourniquet shock and hemorrhage in mice were produced by methods previously described (10). Rectal temperature was measured by an iron-constantan thermocouple.

Oxygen consumption was measured in an apparatus especially designed for small animals. The quantities of oxygen consumed were measured volumetrically at atmospheric pressure. This principle is similar to that used by numerous workers.

The metabolism chamber (fig. 1) for the mouse was a 150 cc. glass cylinder (length: 11 cm.—diameter: 5 cm.) immersed in a large container of water kept in a constant temperature room. Approximately one-half of the volume was filled with soda lime (high moisture content). This was covered with finemesh screen wire, on which the mouse rested. Any errors resulting from contact between urine and soda lime were found to be too small to affect the oxygen determinations. To eliminate this factor completely, the soda lime can be placed above the mouse.

The tube was closed with a rubber stopper through which two glass tubes passed, one to an oxygen cylinder (through a water jar), and the other to the upper end of a 50 cc. graduated gas burette. The opposite end of this burette was connected to a leveling bulb (B), with water as the leveling fluid. An open type manometer containing saline was also included in the metabolism circuit, using a narrow glass tube for the open end.

Before commencing the readings, the system was filled with oxygen at atmospheric pressure. A mark was placed at the surface (A) of the water meniscus on the open tube of the manometer. The gas pressure in the circuit was kept at atmospheric pressure during the experiment by raising the leveling bulb. Readings of the O<sub>2</sub> consumption were then taken on the gas burette every minute.

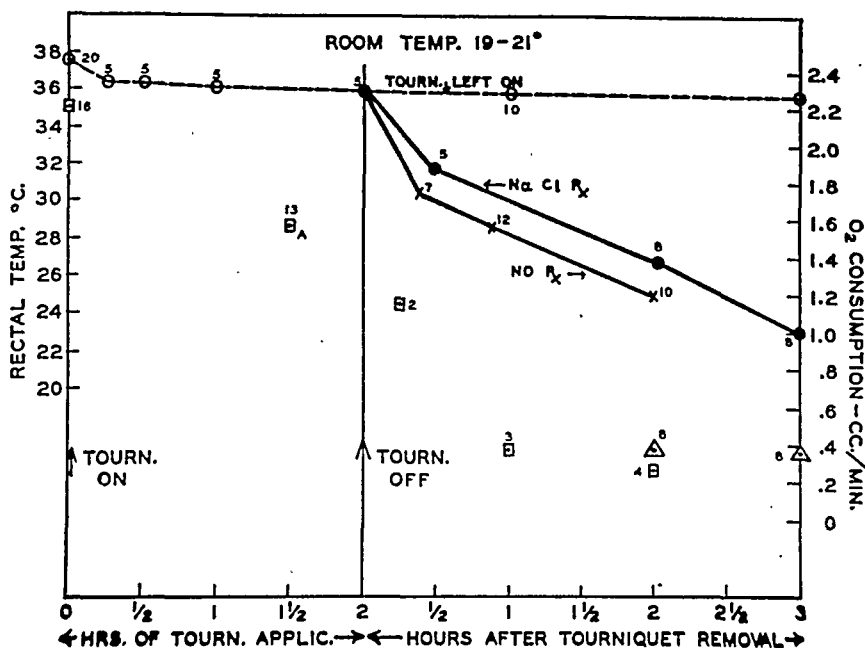


Chart 1. Rectal temperature and  $O_2$  consumption in tourniquet shock at an environmental temperature of  $19-21^\circ$ . The saline treated group received 15 per cent of body weight of 0.9 per cent NaCl intraperitoneally when tourniquets were removed.  $\Delta$  and  $\square$  represent oxygen consumption values for treated and untreated groups respectively. Point A = average of readings at different periods during tourniquet application. Number of mice used at each point is indicated.

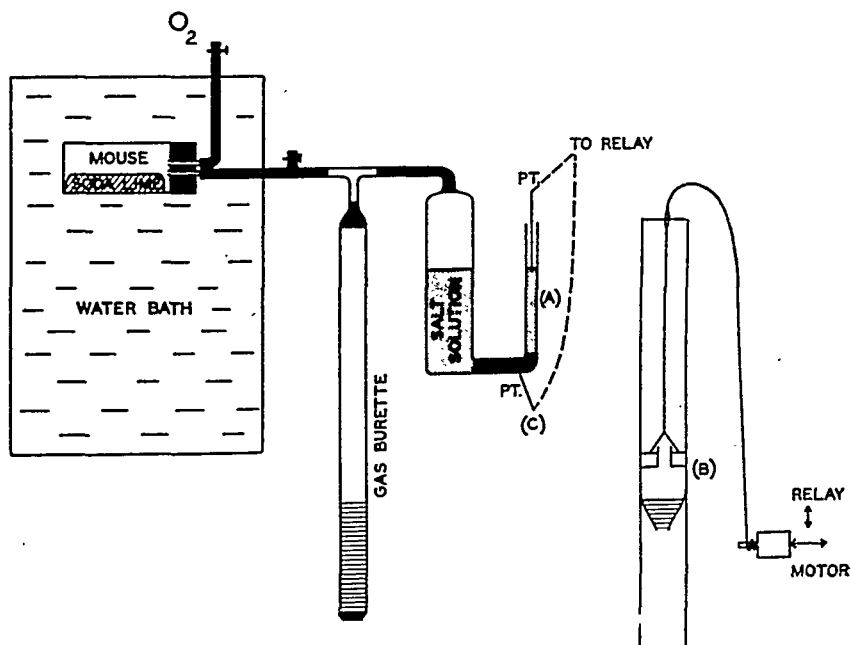


Fig. 1. Apparatus for measuring oxygen consumption of small animals

It is convenient, but not necessary, to raise the leveling bulb automatically. The manometer fluid was composed of concentrated NaCl solution. A platinum electrode was included in the fluid in the U-tube of the manometer (C); another fine platinum electrode was placed so that it just made contact with the fluid surface in the open tube. This operates a relay and motor system when the contact is broken as a result of a decrease in the internal pressure of the metabolic circuit (resulting from O<sub>2</sub> consumption and CO<sub>2</sub> absorption); the motor raises the leveling bulb (B) until the original atmospheric pressure is reached, and contact is re-established.

The quantities of oxygen consumed were corrected to standard conditions of temperature and pressure. All values are expressed as oxygen consumption per 20-gram mouse.

Environmental temperature was controlled by a constant temperature room. For certain experiments requiring several temperatures simultaneously, commercial brooders, electrically heated and equipped with thermoregulators, were adapted for use.

Because of the tendency of mice to huddle together and to nest, individual wire cages free of bedding material were employed. They were constructed of one-eighth-inch wire mesh, 2 by 3 by 3 inches in size, with wire tops.

Female albino mice bred at the Institute, of 16-22 grams' weight, were used. With a few exceptions, food was withheld for 18 hours prior to the experiment, but water was permitted until the application of trauma. The sodium chloride solution employed in all experiments was 0.9 per cent.

*Temperature and Oxygen Consumption in Untreated Mice in Tourniquet Shock.* A depression of body temperature was observed at all environmental temperatures studied (18 to 29°). In experiments done at 18° a moderate decline was noted during the period of tourniquet application (before their release) suggesting that a graded response to injury exists. In 15 mice the temperature fell from 37.3° (S.E. 0.4) to 34° (S.E. 0.2) within 15 minutes after applying tourniquets. Upon release a rapid fall occurred so that within 3 hours the rectal temperature approached that of the environment. Simultaneously, there was a rapid fall in the oxygen consumption. Similar results were obtained at 19 to 21° (chart 1).

*Temperature and Oxygen Consumption in Treated Mice in Tourniquet Shock.* It has been shown that the administration of 10 to 15 percent of body weight of isotonic solutions of sodium salts will prevent death at room temperature from tourniquet shock and other forms of trauma (10).

A repetition of the above experiments in treated mice revealed that saline therapy had little influence on the fall in oxygen consumption and temperature (chart 1). Fifteen percent body weight of saline was given intraperitoneally upon tourniquet removal. At most the only effect seen was a slight retardation in the rate of fall, during the early period following trauma. A similar experiment upon 15 untreated and 17 saline treated mice kept at 29°C. showed in both groups a fall in rectal temperature of only 2° within 2½ hours after tourniquet removal.

Under optimum temperature conditions the temperature and oxygen consumption of treated mice were maintained at levels consistent with survival, but at 18°C. death in hypothermia occurred within 48 hours regardless of the administration of therapy.

It is therefore possible to show that under certain conditions (25 to 29°C.) fluid and electrolyte disturbances can be the major factors in mortality, while

under other conditions (18 to 21°C.) other factors can be demonstrated through their effects on metabolic rate and body temperature.

*Other Therapeutic Procedures.* To investigate the possibility that the metabolic defect might be due to a loss of plasma proteins or to constituents of the blood other than electrolytes, the effects of plasma and whole blood were studied.

*Plasma.* Immediately following tourniquet removal, a group of mice kept at 31°C. were given 2 cc. of saline subcutaneously. Within 3 hours after removal alternate mice received (a) 0.8 cc. heparinized mouse plasma intravenously, or (b) 0.8 cc. saline intravenously, or (c) 0.8 cc. saline subcutaneously. Following therapy they were placed at 19–23°C. The fall in temperature in those receiving plasma was nearly as great as that in the saline treated group (chart 2).

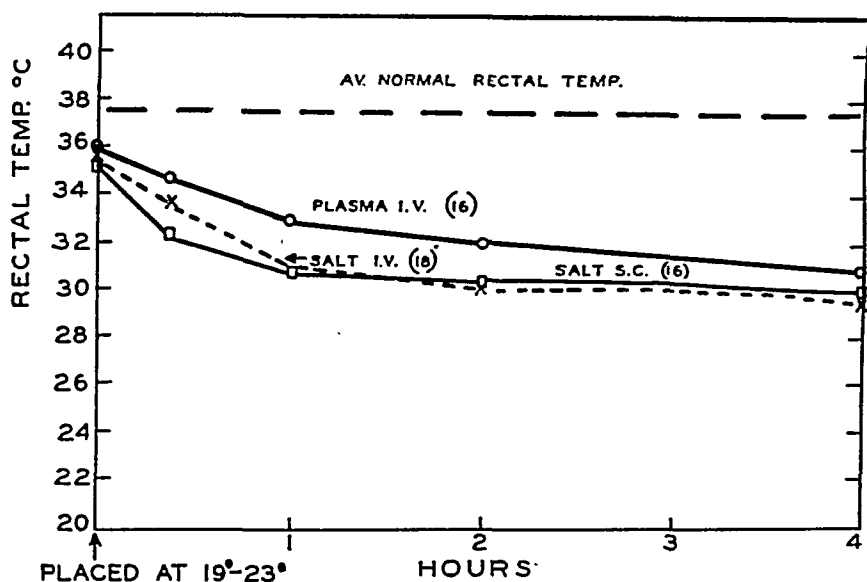


Chart 2. Comparison of plasma and saline upon the temperature fall in shock. Mice received 2 cc. of 0.9 per cent NaCl when tourniquets removed. They were kept at 31°C. for  $\frac{3}{4}$  to 3 hours when additional therapy was given of 0.8 cc. of (a) heparinized mouse plasma i.v.; (b) saline i.v., or (c) saline s.c., and they were placed at 19 to 23°.

*Whole blood.* Heparinized whole blood of mice was diluted with equal parts of saline; 1.5 cc. was injected intravenously into 10 mice upon tourniquet removal. In 5 of them additional injections of 0.7 cc. in one hour and 0.8 cc. in 2 hours were given. The fall in temperature did not differ from that of 17 mice receiving 3 cc. of saline intraperitoneally.

The mice were kept at 18°. Three and one half hours after tourniquet removal the rectal temperature of the saline treated animals averaged 28.5°, as compared with 27.5° for the mice receiving blood.

*Oxygen.* The existence of anoxia in shock is well established (3) (6) (7); it has also been shown that anoxia in normal animals will produce a rapid fall in body temperature which is quickly reversible (8) (9).

Shocked mice (saline treated) were placed in individual wire cages in a desic-

cator jar at 18°, and exposed to 100 per cent oxygen by passing a continuous stream of oxygen bubbled through oil. No difference in temperature fall was observed from that of another group exposed to air under similar conditions (chart 4).

*Whole blood and O<sub>2</sub> therapy on mortality.* The absence of effect of oxygen therapy was also seen in studies with varying oxygen pressures in untreated animals and with whole blood administration upon mortality in burn and tourniquet shock.

Experiments were carried out with oxygen pressures of 58 to 2660 mm. mercury. The decreased pressures were obtained by exposing the mice in desiccators to mixtures of oxygen

TABLE 1

*The lack of effect of oxygen tensions from 58 mm. to 2660 mm. upon survival from tourniquet or burn shock*

Controls represent simultaneous experiments on mice under atmospheric conditions in the same room.

TYPE OF TRAUMA	NO. OF MICE	OXYGEN TENSION	ROOM TEMPERATURE	AVERAGE SURVIVAL TIME	MORTALITY IN 48 HOURS
			°C.	hours	per cent
Tourniquet untreated	20	760 mm.*	22	9	70
	20	controls		6.6	65
	18	760	29	3.3	100
	18	controls		3.3	95
	20	79	22	8.3	90
	20	controls		10.0	80
	20	58	22	5.7	100
	18	controls		14.0	83
Burn untreated	20	760	22	16.0	95
	20	controls		19.0	95
	30	2660	31	1.3	100
	31	controls		1.4	100
	30	2660	19.5	7.2	100
	30	controls		9.1	100
	30	1520	19.5	13.8	100
	29	controls		16.0	100
	20	1520	31	1.5	100
	20	controls		1.5	100

\* 100% O<sub>2</sub> at atmospheric pressure.

and nitrogen. Increased pressures were obtained by the use of a small pressure chamber.<sup>1</sup> These experiments on survival time were run at environmental temperatures from 19.5 to 31°C.

The results are shown in table 1. With the low pressures, some shortening of survival time was observed at 58 mm., but this pressure caused marked dyspneic symptoms in normal mice. In no experiment with increased pressure was a significant prolongation of life brought about. The higher pressures of oxygen were complicated by the fact that they were toxic to normal mice during the period of exposure.

<sup>1</sup> Kindly made available through the courtesy of Dr. I. Gersh of the Naval Medical Research Institute, Bethesda, Maryland.

The absence of effect of whole blood therapy on the mortality in burn and tourniquet shock has been reported previously (10).

Various other procedures have been tried in an attempt to prevent the fall in temperature. The following have been administered without appreciable benefit: Glucose, sodium lactate, sodium succinate, sodium bicarbonate, calcium gluconate, thyroid extract, dinitrophenol, adrenal cortical extract (Upjohn), desoxycorticosterone, metrazol, nikethamide, caffeine, and camphor.

It is of interest that some retardation in the fall in temperature could be demonstrated with ephedrine and amphetamine. Related compounds containing phenolic substitutions, such as epinephrine, tyramine, neosynephrine, and an unrelated sympathetico-mimetic agent, privine, behaved in an opposite way and hastened the fall in body temperature.

*Evidence of local factor (s).* There is general agreement that fluid loss is important in shock, and studies on the sodium deficit and potassium release indicate that they also play a significant rôle (10) (11).

Since the original observations of Cannon and Bayliss (2) on the liberation of toxic factors from traumatized tissues, a large and conflicting literature has accumulated (12). Recent contributions suggest that fluid loss may be inadequate to account for the phenomena of shock (13) (15) (16) (17) (18) (21).

By replacement of clamps upon the legs of untreated rats after an interval of 1 to 2 hours, Haist and Hamilton have shown that certain carbohydrate changes can be reversed, and survival of the animals can occur. Since most of the swelling has occurred within 2 hours they conclude that other factors are involved (13).

Since the fall in temperature and metabolic rate does not respond to blood, plasma, or saline therapy, it was of interest to determine whether unknown local factors from the injured tissues might be involved. Observations were made upon tourniquet replacement in mice that had received adequate saline therapy. The tourniquets were applied initially for 2 hours at 29 to 31°, and they were kept at this temperature until the tourniquets were reapplied, at which time they were placed at 18°, and the temperature curves obtained.

When the tourniquets were never removed, after the initial fall at the time of application, no further decline occurred. Leaving the tourniquets off for 10 minutes produced an additional slight but transitory fall in temperature for 30 minutes; with intervals of 1 and 2 hours before replacement the fall was greater but in both cases recovery occurred after several hours (chart 3).

Another series of observations were made upon tourniquet-shocked (treated) mice in which the animals, following tourniquet removal, were allowed to remain at 31° for varying periods of time before placing them at 18°.

Little protection was conferred by an additional hour at 28 to 31° after tourniquet removal; when the mice were kept at longer intervals at 28 to 31° before exposure to 18°, increasing protection was conferred. With intervals up to 6 hours the responses were variable. In some experiments only a retardation in rate of fall of body temperature was seen; in others the fall was greatly reduced and followed by recovery of some animals within 24 hours. When kept at 28

to 31° for 16 hours, 6 of 13 mice were able to maintain a normal body temperature on exposure to 18°, while with intervals of 32 to 46 hours, 19 of 21 mice survived 18° (table 2).

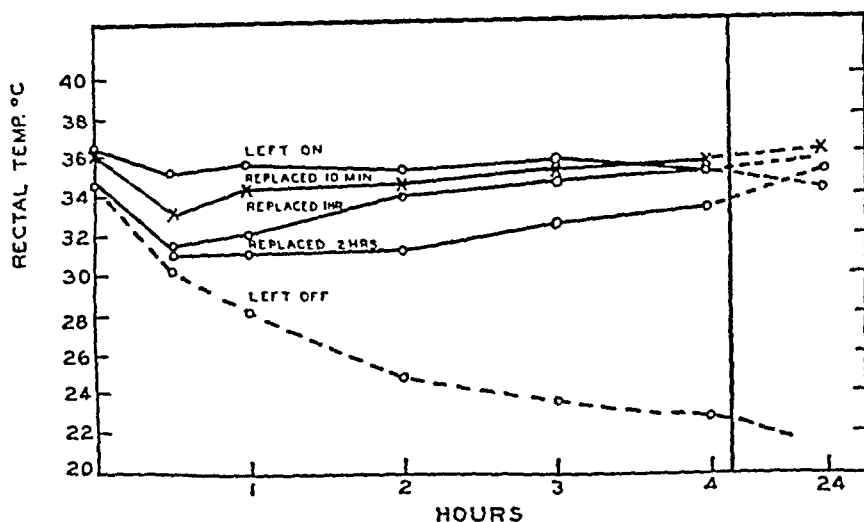


Chart 3. The reversal of temperature fall by replacement of tourniquets after varying intervals. Ten mice in each group. All received saline therapy.

TABLE 2

Temperature fall in shocked mice (saline treated) when kept at 28-31° for various intervals before placing at 18°. The ability to maintain body temperature and to survive at 18° is restored after 16 to 46 hours in a warm environment.

*Average rectal temperature in 18°C. room*

NO. OF MICE	INTERVAL AT 28-31°	INITIAL	½ HR.	1	2	3	4	18-24 HRS.	% MORTALITY 48 HRS.
11	5 min.	35°	32.8	28.2	24.9	23.7	22.9	21.8	100
8	5 min.				21.4		19.8		100
5	5 min.		29.8		23.0		21.4		100
10	1 hr.	35°	30	28.4	26.4	26	25.3	21.6	90
10	2 hrs.	35.7	28.8	28.0			28	30	50
10	3 hrs.	35.2	32.8	30.8	31.2	32		33.2	30
8	3 hrs.		27.8		25.6		25.0	22.0	88
8	6 hrs.							22.2	83
7	6 hrs.							19.8	100
6	16 hrs.	34			30			25.4	67
7	16 hrs.	34		30.8	29.8		28.0	26.2	57
8	32 hrs.							34.6	13
5	32 hrs.							30.8	20
8	46 hrs.						35.0	37.0	0

These experiments indicate that (a) factors from the traumatized legs are responsible for the metabolic and temperature effects. Maximum release of these products does not occur immediately on tourniquet removal, but rather a

gradual absorption takes place over a period of hours. Green and Bielschowsky came to similar conclusions in their studies upon toxic substances in damaged tissues (19). Haist and Hamilton (13) and Meyer, McShan, Goldman, and Shipley (14) have likewise demonstrated the reversibility of carbohydrate and other changes several hours after the injury.

The evidence against fluid loss as a basis for the fall in temperature and  $O_2$  consumption is that the fall can be reversed by ligation of the legs at a time when most of the swelling has occurred, and also that it cannot be influenced by saline or plasma therapy.

Our results further indicate that (b) active neutralization or excretion of such products takes place in treated animals kept in a warm environment. After 3 to 16 hours at 28 to 31°C. the toxic effects, as indicated by the fall of body temperature in an environment of 18°, have partially worn off; and after 1 to 2 days nearly all of the animals can survive at 18°. When the shocked animal is placed at 18° soon after tourniquet removal, the capacity to neutralize or excrete these products is not adequate. This may be due to the rapid fall of temperature and metabolism to levels which interfere with such neutralization or excretion.

Many experiments have been carried out with extracts of normal and damaged tissues, and with various metabolites, in an attempt to identify the local factor. The activity of the substances tested was evaluated by the ability to lower the temperature of normal mice or of mice with a standard amount of trauma (tourniquets to both legs, and allowed to remain on), when placed at 18°. Where activity was present, the substances were also tested on mortality in shocked mice at 28°, following a technique previously reported for potassium and magnesium (10).

Because of the volume of experimental data, it will be said in summary that the activity of the extracts studied, and of certain metabolites appears inadequate to account for the temperature fall in shock. The following metabolites were studied: Potassium, magnesium, calcium, inorganic phosphate, adenine, adenosine, adenylic acid, adenosine triphosphate, histamine, tyramine and acetylcholine.

While Mg and adenosine triphosphate can lower body temperature (19) the quantities required were greater than the amounts present in the tissues.

Potassium possessed little activity in lowering temperature. Evidence has been previously presented that it may be concerned in the mortality from shock (10) but this action is also differentiated from the temperature and metabolic disturbances in that high potassium concentrations are only reached in the later stages of shock.

*Hemorrhage.* In view of the evidence that the declines in metabolism and temperature were due to factors originating at the site of trauma, experiments were done following hemorrhage, where no local trauma is present.

Unanesthetized mice were subjected to hemorrhage with the use of the technique previously described (10). Bleeding was carried out in two stages one hour apart, and 5.5 to 6.0 per cent of the body weight of blood was removed. Since this represents an amount of blood loss which would be fatal to untreated

animals, all mice received 15 per cent of their body weight of 0.9 per cent NaCl intraperitoneally at the end of the first hemorrhage. The experiments were conducted on 83 survivors of a total of 106 animals employed.

Hemorrhage was carried out at a temperature of 26 to 29°. Upon completion the mice were transferred in individual cages to 18°, and the course of rectal temperature observed. A rapid fall occurred, terminating in death in hypothermia, in a manner similar to that of burn and tourniquet shock.

However a clear difference between hemorrhage and trauma was observed in the response to oxygen or whole blood therapy.

The fall in temperature following hemorrhage could be completely prevented by 100 per cent oxygen, and after the rectal temperature had fallen to 21 to 26°, it could be restored to normal by oxygen administration. Similar results could

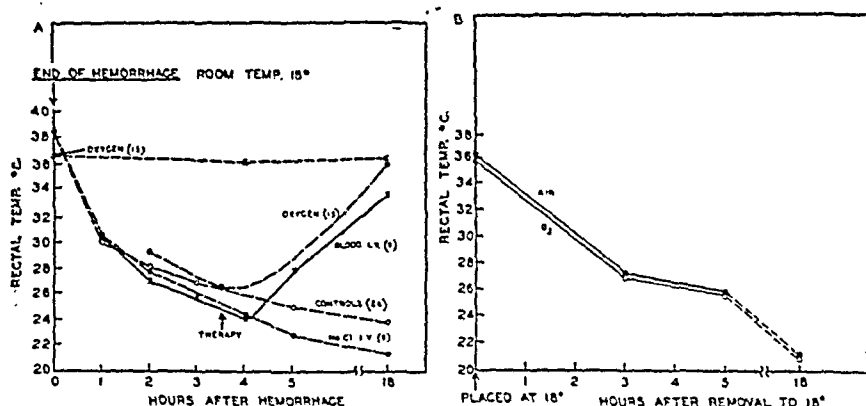


Chart 4 A. The reversibility of the temperature fall following hemorrhage by oxygen or heparinized whole blood (mouse). Lack of effect of saline. All mice received 3 cc. of saline I.P. after first hemorrhage. In the upper curve O<sub>2</sub> was administered when mice placed at 18°; in lower curves O<sub>2</sub> or 0.8 cc. of blood or saline i.v. given 3½ hours later.

Chart 4 B. Lack of effect of 100 per cent oxygen on temperature fall in saline treated mice placed at 18° after tourniquet removal. 15 mice in each group.

be accomplished with intravenous injections of whole blood, while saline intravenously was without effect (chart 4).

These experiments demonstrate that the fall in temperature that occurs in saline-treated animals subjected to hemorrhage is due primarily to anoxia which results from a diminished capacity of oxygen transport. It would seem closely related to the reversible fall that occurs in simple anoxia in normal animals (9).

If the abnormality in traumatic shock were also caused by anoxia, the defect would be in the tissues, since it cannot be corrected by increasing the facilities for oxygen transport.

*The relation of O<sub>2</sub> consumption to body temperature.* It was not possible to conclude from the above results whether the fall in O<sub>2</sub> consumption was primary or secondary to decline in body temperature.

To investigate this the metabolic capacity was tested under the stimulus of a load. Tourniquet shocked mice were kept at 31°, and then their temperature

and metabolic responses were observed upon removing them to a temperature of 18°. All shocked mice were given saline therapy (3 cc. of isotonic saline intraperitoneally, 1 hr. before removal of tourniquets) to obviate as much as possible the influence of fluid and sodium defects.

Normal mice were also subjected to a similar change in environmental temperature, and their rectal temperatures and oxygen consumptions were measured at 18°. Resting values were measured by obtaining readings during periods of inactivity, but the mice did not attain basal metabolic conditions (5). The readings obtained were the responses of normal *untrained* mice to the metabolic load produced by the lower environmental temperature.

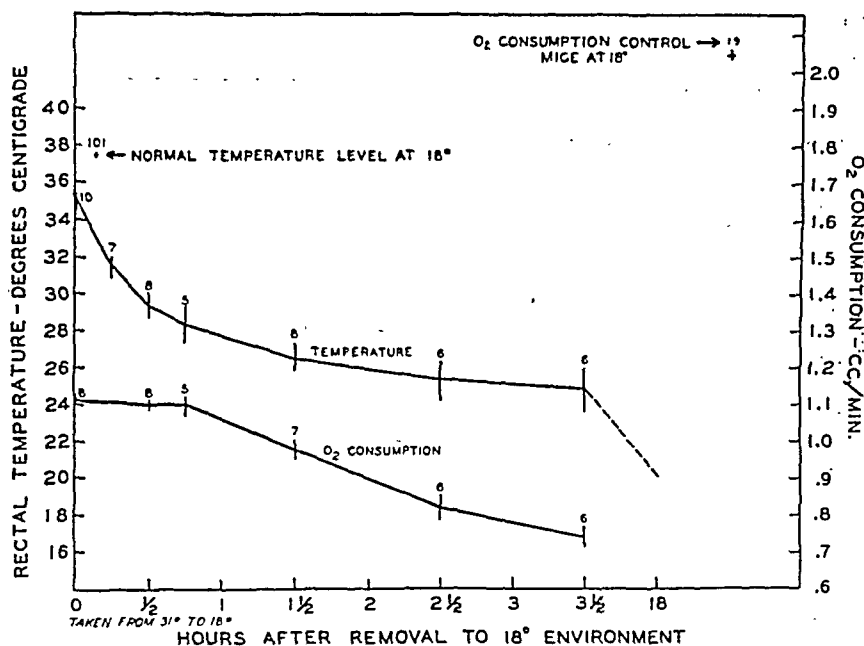


Chart 5. Temperature and O<sub>2</sub> consumption of saline treated shocked mice. One-half to 2 hours after tourniquets removed they were taken from 31° to 18°. Control values obtained on normal mice during periods of observed inactivity; they do not represent basal values. Vertical lines through points represent  $\pm$  S.E. of mean. The number of mice used at each point is indicated.

Normal mice upon removal from 31 to 18° will show an increased oxygen consumption. O<sub>2</sub> consumption values at 18° on 19 mice averaged 2.04 cc., and this rate was maintained during a period of 18 hours' observation. Rectal temperatures in 101 normal mice at 18°, averaged 37.40°, and likewise showed no significant fall during this time (chart 5).

In shocked mice the values first obtained upon removal to 18° (from 31°) averaged 1.11 cc. and this rate was maintained for 30 to 60 minutes even though the temperature began to fall immediately and continued to fall throughout this time. In no case was there a rise in the oxygen consumption above the initial rate observed. After the rectal temperature had fallen to approximately 28°, the oxygen consumption began to decline further (chart 5).

The above evidence suggests that the metabolic *capacity* is reduced approximately 50 per cent in shock. In a warm environment (25–29°) this is sufficient to maintain body temperatures at levels somewhat below normal, but sufficient for survival if adequate fluid and electrolyte therapy is administered. However, on exposure to slight cooling, the metabolic rate is inadequate to maintain body temperature, and as a result a spiralling decline in temperature and metabolism occurs, ending in death of the animal.

*Bleeding volume.* In traumatic shock the metabolic and temperature changes can be brought out most clearly by exposure to a cooler environment. An investigation was made into the existence of other abnormalities that might be demonstrable in the treated animals kept in a warm environment.

As a procedure for studying circulatory impairment in these small animals, bleeding volume was used. The total amount of blood that could be collected following decapitation was determined by pooling the blood from 5 or more animals in glass-stoppered weighing bottles. The procedures were carried out as uniformly as possible, and loss by evaporation was kept at a minimum. The room temperature was 28–29°C. The values are relative, since the amounts collected in normal animals represent approximately one-third of the total volume, but the results under these conditions were sufficiently accurate and reproducible to provide an index of circulatory function (table 3).

Bleeding volume, as a measure of circulatory efficiency, has been found to be greatly reduced in various types of trauma by Harkins (12), Roome, Keith and Phemister (20), and Prinzmetal and Bergman (21). With a type of burn shock characterized by minimum swelling, Prinzmetal and Bergman obtained evidence that the decreased bleeding volume was due to a toxic vascular factor. They further showed that the decrease was greatest when environmental temperatures approached 37°C.

In 29 normal mice not subjected to tourniquets the bleeding volume by the method used averaged 0.55 gram of blood per 20 grams' body weight. In 34 mice with tourniquet application but without treatment the average bleeding volume determined 2 hours after tourniquet removal was 0.170 gram of blood. In 29 mice that received 15 per cent of their weight of isotonic saline intraperitoneally at the time of tourniquet application, the average bleeding volume 2 hours after tourniquet removal was 0.208 gram of blood. A fourth group received an additional 5 to 10 per cent of body weight of saline intravenously when the tourniquets were removed. Even this large amount of therapy did not appreciably restore the bleeding volume, the average of 34 animals being 0.263 gram (table 3).

In order to determine whether plasma proteins or other constituents of plasma might be more effective for this purpose, a series of mice were injected intravenously with 5 per cent body weight of heparinized mouse plasma at the time of tourniquet removal. Alternate mice serving as controls received a similar amount of saline intravenously. Both groups received 15 per cent body weight of saline intraperitoneally when the tourniquets were applied. No effect was observed from plasma; the average bleeding volume of 18 animals was 0.237 gram as compared to 0.275 gram in 26 mice that received saline (table 3).

The lack of response to treatment suggested that factors originating in the traumatized tissues might be important in the production of this circulatory impairment, similar to that observed for the metabolic change. Additional evi-

TABLE 3

*The inability of saline or saline + plasma to restore the bleeding volume to normal in, tourniquet shock*

THERAPY	NO. OF MICE	BLEEDING VOLUME* GMS/20 GMS. BODY WT.
Normal mice		
I. None	6	0.512 gram
	9	0.635
	9	0.536
	5	0.468
	29	0.55 Average
Tourniquet mice		
II. None	5	0.175
	11	0.165
	18	0.171
	34	0.170 Average
III. 0.9% NaCl, 15 per cent body weight intraperitoneally when tourniquets applied	9	0.20
	9	0.233
	4	0.208
	7	0.187
	29	0.208 Average
IV. Saline as in III. (a) + 1 cc. intravenously on tourniquet removal (b) + 2 cc. intravenously on tourniquet removal	6	0.269
	10	0.291
	10	0.267
	4	0.208
	4	0.239
	34	0.263 Average
V. Saline as in III + 1 cc. plasma intra- venously on tourniquet removal	5	0.218
	8	0.227
	5	0.269
	18	0.237 Average

\* 2 hours after tourniquet removal.

dence for this was obtained by estimation of bleeding volume following ligation of the traumatized tissues. A group of animals were treated intraperitoneally with 3 cc. of saline one hour after tourniquets were applied (for 2 hrs.). Follow-

ing an interval of 2 hours after removal of the tourniquets they were reapplied to half the mice. After the lapse of 2 more hours bleeding volumes were determined. The following values were obtained:

Bleeding volume 2 hours after tourniquet replacement:

10 mice = 0.435 gram per 20 grams mouse

9 mice = 0.457

Average = 0.446

Bleeding volume in controls, 4 hours after tourniquet removal:

11 mice = 0.272

10 mice = 0.274

Average = 0.273

Pronounced restoration of bleeding volume was brought about by replacement of tourniquets at a time when local swelling of the legs had approached its maximum.

These results indicate the importance of factors arising in the injured tissues in the production of circulatory impairment. While fluid and sodium depletion can also produce such changes, they can be differentiated by their response to therapy (22). The relative effects of therapy and of ligation of the legs upon bleeding volume suggest that unknown local factors exert the more significant effect. Prinzmetal and Bergman (21) have arrived at similar conclusions from their study of burns.

It is significant to note that the therapy employed can bring about survival at suitable environmental temperatures even though the reduction in bleeding volume remains largely uncorrected during the period of observation.

Since the metabolic and temperature changes show a similar behavior, it is possible that they are secondary to the circulatory impairment, but this has not yet been established.

*Optimum environmental temperature in the therapy of shock.* There is disagreement in the literature on the temperature best suited for survival in shock. Earlier experience, largely clinical (2), led to the use of heat. More recent experimental evidence is in agreement that an environmental temperature below 8°C. or above 37°C. is harmful (23). The optimum range has been variously placed at 16 to 26° (23). The basis for these conclusions has been the survival of untreated animals.

We have repeated the experiments on survival of tourniquet-shocked mice in the range of 18 to 37°C. and have found that the optimum for adequately treated mice is entirely different from that of untreated animals.

In untreated mice the longest survival time occurs at 18 to 20°C. At these temperatures the mice die in hypothermia, and the prolongation of life is at the expense of the low body temperature and metabolism. This prolongation may be unfavorable to the ultimate recovery from the shock state (23); it has been shown above that under these conditions the body is less able to dispose of the factors arising locally.

This is borne out by mortality studies in saline treated animals. Survival

was greatest in the neighborhood of 25°C. and few animals survived at 18 to 22°C. (chart 6).

The unfavorable results of a cool environment are not to be confused with the beneficial effects of local cooling of the traumatized areas, first demonstrated by Allen (24).

At 31°C. the harmful effects of heat also begin to appear in the treated animals (40 per cent mortality). In contrast to the deaths at lower temperatures, these occur within the first few hours, indicating increased production or increased toxicity of the factors involved.

Experiments which we have done at 37°C. indicate that these factors originate

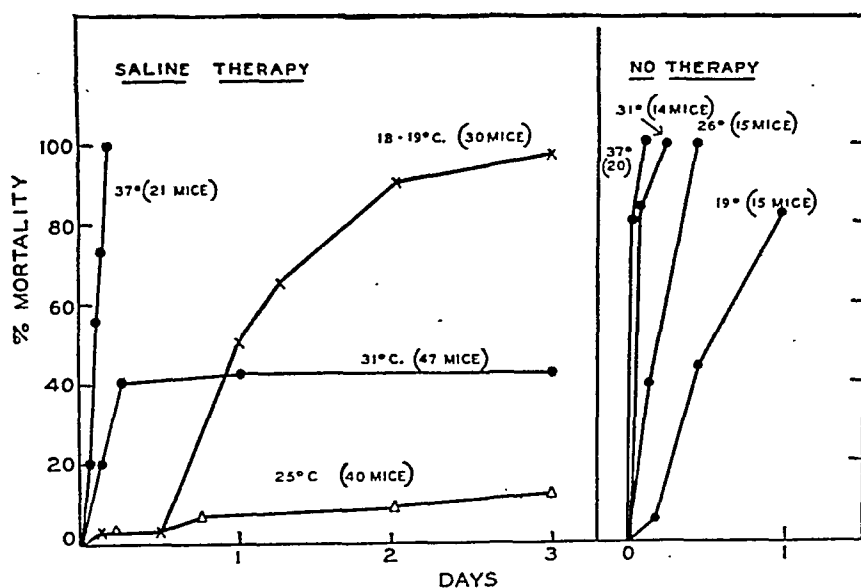


Chart 6. The mortality curves of treated and untreated tourniquet-shocked mice at different environmental temperatures. Therapy was 15 per cent of body weight of saline intraperitoneally, before tourniquet removal. The optimum for treated mice is in the neighborhood of 25°C. The longest survival in untreated mice occurred in the neighborhood of 19°. Numbers of mice used shown in parentheses.

in the injured tissues, similar to those involved in the response to cooling. The harmful effects of heat are refractory to saline therapy, but can likewise be combatted by ligation of the injured areas (tourniquet replacement).

It is thus seen that in mice adequately treated with saline, when kept in individual cages the maximum therapeutic response occurs within the relatively narrow range of 25 to 29°C., beyond which the harmful effects of heat and cold appear.

Apart from therapeutic implications, these studies show the need for accurate temperature control in experimental studies on shock. At certain environmental temperatures the effects of these local factors are so great that they may obscure or make difficult the study of other processes, such as fluid and electrolyte disturbances.

DISCUSSION. The above observations may aid in clarifying some of the confusion that exists in the mechanism and treatment of shock.

In traumatic shock at least two processes seem to be involved: fluid and electrolyte disturbances and unknown local factors. While the changes they produce in the body may be interrelated, it is possible to differentiate them on a basis of response to therapy.

Either process can be adequate to produce death. It has been shown that therapy directed to correction of the fluid and electrolyte changes has little influence on the reactions in the body produced by the unknown local factors. Under the conditions of our experiments correction of the former changes can bring about survival if the animal is kept at a suitable environmental temperature. However, if additional demands are placed upon the circulation and metabolism by a temperature too high or too low, death can result. No successful treatment has been found to counteract the local factors, although ligation of the traumatized areas will abolish their effects. These two aspects of shock, the one responsive to available treatment, and the other refractory, may contribute to a better understanding of the two states termed "reversible" and "irreversible" shock.

Under the conditions of our experiments the principal factors involved in death from hemorrhage appear to be fluid and electrolyte loss and an inadequate oxygen transport system. Our results suggest that along with whole blood and saline administration, oxygen therapy may be of value in hemorrhage.

Circulatory tests are frequently used to evaluate the effects of therapy in traumatic shock. Unknown local factors would seem to play an important rôle in the circulatory impairment; with the use of bleeding volume as an index, it has been found that the administration of plasma and electrolytes has slight effect in correcting this disturbance.

Even though bleeding volume is not restored by administration of saline or plasma, this therapy can bring about a large percentage of survival. Therefore, circulatory tests, as exemplified by bleeding volume, may not afford a satisfactory index of the benefit of such therapy.

#### CONCLUSIONS

A fall in metabolic rate and in temperature has been demonstrated in traumatic shock in mice. Body temperature approaches room temperature, and death in hypothermia can be brought about at an environmental temperature of 18-22°C.

An impairment in circulation, as indicated by a diminution of bleeding volume to 30 per cent of normal, has been shown.

Evidence is presented that these disturbances are a result of factors originating in the injured tissues. Available therapeutic measures have little effect upon them, but ligation of the injured tissues, even after several hours, will correct them.

The fall in temperature following hemorrhage has been shown to result from a diminished capacity for oxygen transport. The temperature can be restored by oxygen or by whole blood therapy.

In tourniquet-shocked mice that have received adequate therapy the optimum temperature for survival is 25 to 29°C. At these temperatures the majority of mice will survive an otherwise fatal trauma if they receive 10 to 15 per cent of their body weight of isotonic NaCl. Outside this temperature range the harmful effects of heat and cold appear; they are related to factors arising from the injured tissues and are little influenced by available therapy.

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# THE PERIPHERAL VASCULAR SYSTEM AND ITS REACTIONS IN SCURVY: AN EXPERIMENTAL STUDY<sup>1</sup>

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The morphological pathology of scurvy has been well established in supporting tissues and in various organ systems (1, 2, 3), but there is a disagreement with regard to the status of the capillary bed. Although characteristic lesions have not been described in the capillaries (4), several studies of experimental and of clinical vitamin C deficiency have reported an "increased capillary fragility" in this condition (5, 6, 7, 8). Increased vascular fragility has been suggested, with certain limitations, as an early criterion of ascorbic acid deficiency, since it appears prior to the onset of other characteristic signs of the scorbutic state (6). Other workers, in studies chiefly of human vitamin C deficiency, have found no significant correlation between blood levels of ascorbic acid and the tendency to petechiae formation (9, 10, 11, 12, 13).

In view of this lack of agreement, and the absence of information concerning the physiology of the peripheral vascular bed in scurvy, it was decided to make direct microscopic observations of the reactions of the smallest blood vessels in living scorbutic animals. Within the past few years, methods for the study of the capillary bed in living mammals have been considerably improved and found suitable for the establishment of peripheral vascular phenomena in several different experimental conditions (14, 15, 16, 17). In the present study, these methods have been slightly modified and adapted to the examination of the terminal vessels in living unanesthetized control and scorbutic guinea pigs.

**METHODS.** The procedure involves observation with the microscope of the mesenteric capillary bed in guinea pigs which are not under a general anesthesia.

1. *Preparation of the Mesentery.* Animals were prepared for examination by a paravertebral block of the lower thoracic and upper lumbar nerves, using a total of 0.4 cc. of 1.0 per cent Procaine solution. In six animals, these nerves were sectioned surgically two weeks prior to study. For the microscopic observations, the animals were fastened into a suitable device which prevented them from moving about, and the abdominal wall was incised across the denervated or blocked area. Respiratory movements of the abdomen slowly extruded a gut loop which was gently guided with its mesentery over a movable glass ring on the microscope stage. The tissue was warmed by irrigation with a

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Ringer-gelatin solution at 37.5°C. and completely covered except for the region studied by cotton strips soaked with the warmed drip solution. Observations and readings were taken and checked. This loop of mesentery and gut were then pushed aside. The slow extrusion of gut loops through the incision by respiratory movements afforded new fields for study. The mesentery was never stretched prior to observation.

At no time during these studies did the animals exhibit any evidence of pain. The avoidance of general anesthesia, and the absence even of a local anesthetic in six animals, made possible the observation of the splanchnic vascular bed in a living, fully conscious mammal. This ruled out the possibility of an increased sensitivity in scurvy to the vaso-inhibited state which has been demonstrated following the use of some of the common anesthetic agents (17).

2. *Vascular Criteria.* In all animals, the following data were recorded: the presence or absence of spontaneous vasomotor activity, the diameters of the capillaries and the nature of their wall, the general state of capillary blood flow, the presence or absence of petechiae, and the presence or absence of any vascular phenomena following violent struggling by the animal. In addition, the threshold concentrations of epinephrin were determined which, when applied topically to the mesentery, produced complete closure of pre-capillary sphincters and contraction of arterioles to approximately one-half their diameter. This offered a quantitative measure of the reactivity of these muscular elements to an external stimulus. In 4 controls, and 3 scorbutic animals, studies were also made of the epinephrin reactivity of the small venules.

3. *Experimental Procedure.* A. Series A. *Controls.* Thirteen animals were used; ten were prepared by a Procaine nerve block and three by operative denervation of the flank two weeks prior to study. Nine animals were given a diet (diet A) known deficient in ascorbic acid (18) with greens *ad-libitum* to insure adequate vitamin intake. Four were given a purified ascorbic acid deficient diet (diet B<sup>3</sup>) with additional feedings of this vitamin, 15.0 mgm./day. Animals on either diet, supplemented by greens or by oral vitamin C, have been maintained for varying periods of time up to six months or more without evidence of any deficiency state, and have bred to the second generation (19).

*Scorbutic.* Sixteen animals were used; thirteen were prepared for observation by Procaine nerve block, and three by denervation. Twelve received diet B and four received diet A, without supplemental feedings. Studies on both the controls and the scorbutic animals were carried out during the fourth week on their respective diets. At this time, those guinea pigs on the scorbutogenic diets all showed noticeable evidence of scurvy, and their weights had fallen to levels present at the beginning of the diet (200–250 grams). The control animals had no evidence of scurvy or other disease state.

Irrespective of the type of diet or the use of Procaine or surgical nerve block,

<sup>3</sup> Diet B contained: casein 18 per cent, dextrin 45 per cent, salts (S.M.A. Co.) 4 per cent, lard 8 per cent, sucrose 15 per cent, agar 2 per cent, C.L.O. 1 per cent, W.G.O. 2 per cent, yeast 5 per cent. The diet was made in bulk weekly and refrigerated. Adequate daily portions were fed *ad-libitum*. Control animals received an additional daily feeding of ascorbic acid, 15 mgm./100 grams body weight.

the peripheral vascular findings in the control animals were essentially the same. Similarly, regardless of the type of scorbutogenic diet received, or method used to permit exposure of the mesentery in unanesthetized animals, the vessels of the scorbutic animals approached an equal degree of dysfunction in all cases.

B. Series B. After completion of the first series of observations, the following experiments were made. Seven animals on diet A with abundant greens for one week were examined as controls, and the abdominal wound closed following the study. After a two week healing period, these same animals were then placed on scorbutogenic diet B until symptoms of scurvy appeared, when they were examined a second time. The results of this series were completely in accord with those of the first, and offer comparison of both control and scorbutic findings made on the same animals.

RESULTS. *The control animals.* The general topography of the capillary bed in the guinea-pig mesentery agrees closely with that observed in the rat and dog (13) (fig. 1). The arterioles were tonic and showed a threshold response to epinephrin with average concentrations of 1:1,000,000. Their walls were generally thickened, smooth muscle cells stood out sharply in partial contraction, and the vessels showed the intermittent vasomotor activity previously described in the dog and rat (13).

The pre-capillaries reacted to 1:2,000,000 concentration of epinephrin, on the average, but the variability at this region was extreme. In two animals, not included in the data because of their obvious hyper-excitability, complete pre-capillary closure occurred at 1:35,000,000 epinephrin. Vasomotion of the pre-capillary sphincters was usually active, and predominantly in the "closed" or constricted phase. Capillary blood flow was intermittent.

The true capillaries had an average diameter of 7.5-10.5 micra, with the distal third frequently 1.0-1.5 micra wider than the proximal portion. At no time were active contractions found in the capillaries, other than at the pre-capillary region where smooth muscle cells occur.

The small collecting venules appeared partially narrowed, and blood flow through them was rapid. Their epinephrin reactivity was generally comparable to that of the arterioles, although their contracted state was of a more varicose nature in contrast to the more uniform contractions of the arterioles.

The absence of a general anesthetic afforded study of the changes in the small blood vessels of the mesentery and serosa during occasional periods of excitement with violent muscular activity. Such vascular phenomena were seen in six of the twenty control animals. Briefly, when marked, these changes consisted of narrowing of arterioles and a complete closure of pre-capillary sphincters, with restriction of blood flow only to the direct A-V channels. Flow in all of the capillaries and in many of the small arterioles was stagnant. This sudden cessation of flow in many of the larger arterioles implied a contraction of vessels in the arterial tree located more proximally than those vascular beds examined in this study. When muscular activity ceased, the arterial flow gradually resumed; but it was frequently 30 to 60 seconds before capillary blood flow was restored to its previous level, in the mesentery and serosa.

Prominent lymphatics accompanied the larger arterioles and small arteries,

and frequently showed peristaltic contractions at a rate of 1 to 3 per minute. The addition of washed red cells to the Ringer-gelatin drip solution bathing the mesentery was followed by the appearance of red cells inside these lymphatics within 2 to 4 minutes. Forward movement of fluid containing these cells occurred only during the contractions of the lymphatic vessel. Topically applied epinephrin, 1:500,000, was followed by a spastic constriction of the lymphatics to approximately one-third their initial diameter, with a complete cessation of lymph flow. Weaker dilutions produced a speeding of the rate of periodic contraction to as many as 10 to 20 per minute, with varying degrees of constriction observed in the same lymphatic vessel.

*The scorbutic animals.* The peripheral vascular system was generally dilated, and blood flow was markedly slowed. The largest arterioles, of a diameter

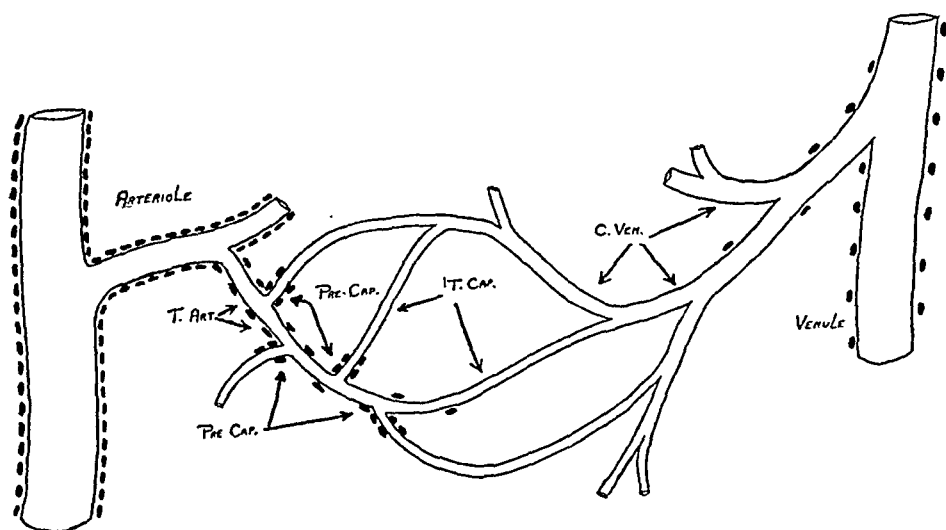


Fig. 1. Diagram of a functional unit in the peripheral vascular bed of the guinea pig mesentery. *T. Art.* = terminal arteriole; *Pre-Cap.* = pre-capillary; *T. Cap.* = true capillary; *C. ven.* = collecting venule. Smooth muscle cells are indicated by the black dots.

greater than 100 micra, showed an undiminished response to topical epinephrin (table 1). In contrast, the direct branches of such vessels, of approximately 30 to 60 micra in diameter, and the smaller arterioles supplied by them, were completely unresponsive to epinephrin, even when relatively strong concentrations were used (table 1). During the notable contraction of the larger parent arteriole in the epinephrin test, the rate of flow through these dilated side branches and the vascular beds supplied by them was greatly reduced, becoming sluggish and frequently stagnant.

The pre-capillary sphincters likewise did not respond to the usual concentrations of epinephrin used. They remained opened (7.0 to 9.0 micra wide) in approximately 90 per cent of the vessels observed (figs. 2 and 3). Spontaneous vasomotor activity was absent; the capillary flow was therefore continuous, but slow. We observed no abnormalities of the capillary wall; and capillary

diameters in scorbutic animals were the same as those found in the controls (table 1).

An especially prominent feature of the scorbutic state was the dilatation and sluggish flow observed in the small collecting venules. The dilatation often involved the venular end of the true capillary before it joined with a venule. The possible importance of this atonic state of the small venules with regard

TABLE 1

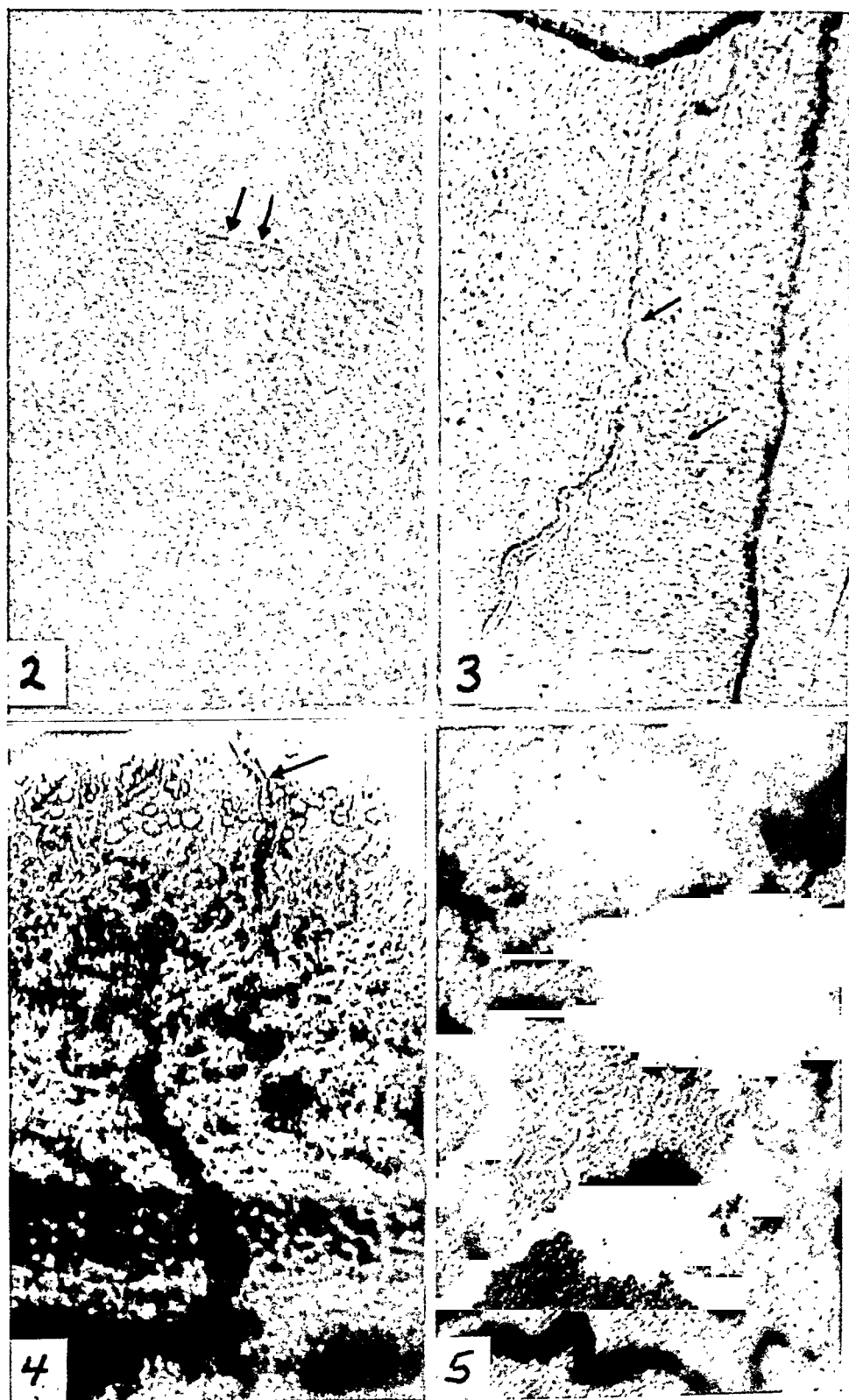
	CONTROL ANIMALS	SCORBUTIC ANIMALS
Epinephrin sensitivity of larger arterioles,* >100 $\mu$ in diam.	1:500,000 (1:100,000-1:5,000,000)	1:450,000 (1:100,000-1:4,000,000)
Epinephrin sensitivity of smaller arterioles,* <75 $\mu$ in diam.	1:1,000,000 (1:300,000-1:5,000,000)	No responses ever noted using 1:100,000
Epinephrin sensitivity of precapillary region**	1:2,000,000 (1:500,000-1:35,000,000)	No responses ever noted using 1:100,000
Epinephrin sensitivity of small venules,* <75 $\mu$ in diam.	1:500,000 (4 animals)	No responses noted using 1:100,000 (3 animals)
Capillary diameter (external), in micra	7.0-10.5	7.0-11.0
Presence of vasomotor activity in arterioles and precapillaries	+ - + + + +, Usually in the "closed" phase	None observed; precapillaries usually opened widely
General nature of blood flow in the arterioles, capillaries, and venules	Rapid, varying with vasomotion; vessels "tonic"	Sluggish, vessels usually dilated, especially in small collecting venules
Presence of petechiae in small venules following trauma	Three in 2 of 20 animals	Present in 11 of 23 animals; numerous

\* Epinephrin concentration necessary to produce narrowing to approximately 50 per cent of internal diameter.

\*\* Epinephrin concentration necessary to produce complete closure of the vessel at this site.

to petechiae formation will be mentioned in the discussion. The epinephrin reactivity of the venules was found to be greatly diminished in the three animals so tested.

The pronounced vascular response to violent struggling found in six control animals was not observed in any of the scorbutic cases, although they likewise struggled and attempted to free themselves as frequently and as actively as did the controls. In two instances, however, the struggling episodes occurred



Figs. 2-5.

fortuitously as epinephrin, 1:200,000, was applied to the mesentery. Within two seconds after the onset of muscular activity, all of the arterioles and pre-capillaries in the field under study showed marked constrictions with a complete stagnation of flow. This exaggerated constrictor response faded soon after struggling had ceased. The heightened responsiveness to epinephrin during these episodes of activity probably represents a vascular response to a summation of intrinsic stimuli (increased concentration of epinephrin in the blood?) with the topically applied vasoconstrictor agent, indicating that in marked scurvy the vascular bed is capable of maximum constriction provided that the stimuli are of adequate magnitude.

*Peripheral vascular hemorrhages in scurvy.* Petechiae rarely appeared spontaneously in the small vessels of the control or the scorbutic animals. In the latter, however, brushing the area observed with a small camel hair brush for ten strokes with a force just ample to stretch the mesentery slightly, or purposefully stretching the mesentery with rubber-tipped forceps, produced numerous small petechiae in eleven of the twenty-three scorbutic animals so treated. The same procedure produced three petechiae in only two control animals. Although the method is not sufficiently quantitative, it suggests that in scurvy there is an increased tendency of the dilated vessels to rupture following trauma.

The petechiae produced in this manner occurred chiefly in the venular portion of the peripheral vascular bed, where vasodilatation and the slowed rate of blood flow were most marked (figs. 4 and 5). For example, of eleven observed in one area, nine were in the small collecting venules which directly drain the capillary bed, one was in a true capillary, and one was in a small arteriole at a point of dichotomy. Similar distributions of petechiae were noted in the remaining ten scorbutic animals in which they were found.

**DISCUSSION.** The disfunction of the peripheral vascular apparatus in scurvy, as found with these methods, displays at least two prominent features. These are:

1. A decreased responsiveness of the contractile elements, particularly of the arterial portion beyond the pulsatile small arteries, to physiological concen-

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Fig. 2. Normal animal: an arteriole, giving off a capillary (arrows); in an environment of epinephrin, 1/5,000,000. Note that the lumen of the capillary is empty, due to contraction of the precapillary sphincters in this concentration of epinephrin.

Fig. 3. Scorbutic animal: a terminal arteriole giving off two capillaries; in an environment of epinephrin, 1/150,000. Note that both capillaries are filled with a moving stream of blood, due to failure of the precapillaries to contract in response to this concentration of epinephrin.

Fig. 4. Scorbutic animal: the venular ends of capillaries are visible in the top of the picture, and are intact, without petichiae. In the center of the figure, the small venules draining the capillaries are partly surrounded by small hemorrhages, as dark masses. At the bottom of the picture, two larger masses of blood are seen outside the main collecting venule.

Fig. 5. Scorbutic animal: larger petechiae are shown here, along the tributary branches of the collecting venules.

(All magnification is approximately 120X)

trations of epinephrin, with dilatation of these muscular vessels and a relatively sluggish flow of blood.

2. A tendency of the terminal collecting venules, which drain the capillary bed, to become dilated and engorged, and to rupture at trauma.

Although these phenomena are probably closely related, they will be discussed separately.

1. The means by which peripheral vascular hypotonia develops in scurvy is obscure. It may be a direct impairment of the ability of the vascular smooth muscle cells to respond to stimuli. However, when epinephrin was applied to the mesentery of an active animal, or was used in very strong concentrations, all vessels constricted maximally. This indicates that the inherent contractile ability of these cells is not notably disturbed. The hypotonia may therefore represent an indirect result of ascorbic acid deficiency, from the dysfunction of some vasotonic mechanism(s), such as the adrenal gland. Adrenal vitamin C is markedly lowered in scurvy (3). The studies of Sayers and his collaborators indicate that this vitamin may prove instrumental in adrenal cortical function (20, 21). Lowenstein has recently isolated an ascorbic acid-cholesterol complex from aqueous adrenal extracts which has definite cortical activity (22). Giroud has concluded that the elaboration of adrenal cortical hormones is dependent on the presence of vitamin C (23, 24). These findings suggest that during ascorbic acid insufficiency, certain aspects of adrenal function may be depressed.

It would be possible to explain the vascular hypotonia of scurvy, in part at least, on the basis of such a scorbutic impairment of adrenal cortical activity. Swingle has shown that the cortex is necessary to maintain a normal vaso-compensatory resistance to hemorrhage (25). After numerous recent experiments, Zweifach and his co-workers have concluded; "Vascular tone, and specifically that of the terminal arterioles, is directly dependent on the presence of cortical hormones. In adrenalectomized rats, the tone and reactivity of these muscular components are markedly depressed. The administration of cortical extracts to such animals results in the restoration of normal vascular reactions" (26). Therefore, the hypotonic state of the vascular bed in scurvy may result from an inadequate function of the adrenal cortex, or perhaps other vasotonic mechanisms.

Scorbutic guinea pigs are killed by a degree of Noble-Collip drum trauma which is readily survived by control animals (18). In addition, the development of resistance to such injury which appears in normal animals after repeated sub-lethal damage, cannot be produced in the vitamin C deficient guinea pigs (18). It has been shown previously that death produced by the Noble-Collip drum is preceded by a hypo-reactive, atonic peripheral vascular system (27). The development of resistance to such trauma, on the other hand, is accompanied by a greatly heightened vasomotor tone. Epinephrin reactivity is markedly elevated, and spontaneous vasomotion is increased in occurrence and in rate (28). This hyper-reactive vascular state is in direct contrast to that obtaining in ascorbic acid insufficiency. Therefore, it is conceivable that the failure of scorbutic animals to withstand trauma or to develop resistance to it is inti-

mately related to their observed decrease in peripheral vascular reactivity, and a consequent impotence of vaso-compensatory mechanisms. Both conditions, however, may result independently from the widespread pathological changes which occur in scurvy (3).

Recent studies have offered evidence that pretreatment with ascorbic acid is of value in protecting guinea pigs against trauma (29, 30) and against hemorrhagic shock (31).

2. Vascular hemorrhages in scurvy, in this study, were found to result chiefly from traumatic rupture of the proximal collecting venules which drain the capillary bed directly (figs. 4 and 5). Dilatation and a sluggish rate of blood flow were most marked in these vessels. It is of interest to note that aggregations of collagen fibers first become notably apparent about the vessels in this region where the true capillaries empty into the venules. Smooth muscle cells are usually absent at this site in the guinea pig, and also in the frog (32). It has been suggested that hemorrhages in scurvy result from a weakness of either the endothelial cement or the collagen substance immediately adjacent to the capillaries (33). Our observation that the great preponderance of hemorrhages occur in the small venules about which collagen bundles are present, rather than in the true capillary endothelial tubes where such collagen aggregations are least prominent, tends to support a weakening of the collagen as a causative factor here, rather than any fault in the capillary endothelium or its cement substance. Chambers has concluded that in certain epithelial tissues ascorbic acid is not essential for the maintenance of the cement material (34). The defect in the perivascular supporting structures of the venules may be closely related in some manner to the greatly slowed flow of blood through these vessels.

An increased tendency of small vessels to rupture at trauma is not a specific response to ascorbic acid deficiency (13). Formation of petechiae was noted in the dilated vessels of the dog's omentum during the depressed terminal stage of "irreversible" hemorrhagic shock (15). Here they were readily produced by light trauma, and occasionally developed when trauma did not occur. They were not found in the capillary bed during its tonic stage prior to hemorrhage or during the early "hyper-reactive" stage of shock. Therefore, it is possible that the increased tendency of small vessels, especially venules, to rupture may be a function of their atonic dilated condition and the relatively slowed flow of blood through them.

The absence of spontaneous petechiae in these experimental studies agrees with the findings of Farmer and his collaborators in a recent clinical study of experimental human ascorbic acid insufficiency (11). These workers report small hemorrhages about wounds, which were probably produced by light surgical trauma. Petechiae were not found in other areas.

#### SUMMARY

1. A technique was devised which permitted study of the small vessels in the mesentery of unanesthetized guinea pigs.

2. The primary pathological condition in the peripheral vascular system of

scorbutic animals was found to be a hyporeactivity of the contractile vessels with dilatation, and a sluggishness of blood flow. This state was marked in the small terminal venules.

3. These conditions developed only in the small vessels distal to the pulsatile arteries and arterioles of approximately 100 to 150 micra in diameter. The responses of vessels larger than this range to topical epinephrin tests were within normal limits.

4. Following trauma, petechial hemorrhages were found in eleven of twenty-three scorbutic animals. They were present to a slight degree in two of twenty controls.

5. At least 85 per cent of the petechiae were located in the small collecting venules which drain the capillary bed directly.

6. In the scorbutic animals, the capillaries were of the same diameter as those of the controls. No abnormalities of the capillary wall were observed.

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# THERMAL BALANCE OF MEN WORKING IN SEVERE HEAT

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In our studies of physiologically equivalent conditions of air temperature and humidity one end point of heat tolerance used was the determination of the hottest environments in which men working at a standard rate could maintain thermal equilibrium during exposures of 6 hours' duration (Robinson, Turrell and Gerking, 1945). A number of 6-hour experiments were carried out on each of 6 subjects to determine the end points of heat stress in both humid and dry environments. From the data of these experiments Gerking and Robinson (1946) reported that without exception when the men's initial rates of sweating were 1.2 kgm./hr. or more there were declines varying from 6 to 75 per cent in their rates of sweating by the 6th hour of the exposures. This confirmed the report of Johnson, Pitts and Consolazio (1944) who observed a decrease in the rates of sweating of men during prolonged work experiments in hot environments. The purpose of the present paper is to analyze the effects of this failure of the sweating mechanism on the heat balance of the subjects.

**PROCEDURE.** Men in good physical condition and well acclimatized to work in the heat were used as subjects in this study (table 1). The experiments were carried out in an air conditioned room in which air temperature and humidity were maintained constant during each exposure. The air movement was 55 m./min. in all experiments. In conditions designated as humid in this paper the dry bulb temperature was 32 to 35°C. with corresponding wet bulb temperatures of 31 to 33°C. Under these conditions the average radiating surface temperature of walls, floor, ceiling and objects surrounding the subject was 0.3 to 0.4°C. below air temperature. In dry atmospheres where the air temperature was 50°C. the surrounding surfaces had an average temperature of 1.3°C. below air temperature. The standard rate of work consisted of walking on a motor driven treadmill at 5.6 km. per hour up a 2.5 per cent grade (average metabolic rate 190 Cal./m<sup>2</sup>/hr.). In a few experiments the men walked on the level at 4.5 km./hr. (average metabolic rate 125 Cal./m<sup>2</sup>/hr.). The experiments reported here were all of 6-hours' duration. During all experiments the men kept their body weights constant by drinking at frequent intervals measured quantities of 0.1 per cent sodium chloride solution which was kept at a temperature of 35 to 37°C. This

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concentration of salt was acceptable to the men and served to replace in large measure the salt lost in sweat, thus helping to maintain body fluid volumes. In some experiments the men wore only shorts, shoes and socks while in others they wore in addition Army tropical uniforms made of a lightweight windbreak poplin fabric. During each exposure the following observations on the subjects were made: (1) Rectal temperature was measured by clinical thermometer at 30-minute intervals. (2) Skin temperature was measured at 15-minute intervals by four thermocouples placed separately on knee, chest, hip and shoulder. These points are best for working subjects because the thermocouples will remain in place for hours. The values of skin temperature presented in this paper are averages of the 4 measurements made during each hour at these 4 points. (3) Rate of sweating was calculated from the nude weights of the men taken immediately before starting and at the end of each hour of the experiment, taking into account metabolic weight loss, the water intake of the men and urine output if any was voided during the experiment. (4) Rate of evaporation was calculated in the same manner from weight changes of the clothed subjects. The subjects

TABLE 1  
*Physical characteristics of the subjects*

SUBJECT	AGE	HEIGHT	WEIGHT	BODY SURFACE
	<i>Yrs.</i>	<i>cm.</i>	<i>kgm.</i>	<i>M<sup>2</sup></i>
M. S.	23	186	72.5	1.97
S. R.	41	171	65.5	1.76
L. G.	23	175	62.5	1.76
A. J.	26	181	67.5	1.86
B. S.	24	182	69.2	1.88
Av.....	27	179	67.4	1.85

stopped walking for five minutes at the end of each hour for the weighings. (5) In a number of experiments the metabolic rates of the subjects were measured by collection and analysis of expired air.

RESULTS. Figure 1-A shows the effects of two intensities of work on the heat exchange of two men. The subjects, S. R. and M. S., were both thoroughly acclimatized and about equally capable of regulating body temperature under equal conditions of work and heat stress. In the experiments shown in this figure both subjects wore the poplin tropical suits, and both walked for 6 hours in an environment where the air temperature was 50°C. and the wet bulb temperature 28°C. Only the work was different for each man. Subject M. S. walked on the treadmill at 4.5 km./hr. (M.R. 125 Cal./m<sup>2</sup>/hr.) and his body temperature remained practically unchanged from the 2nd through the 6th hours of the exposure. His rate of sweating declined during the last 4 hours, but evaporating the sweat secreted during this period plus excess sweat he had accumulated in his clothing during the first two hours made his total rate of evaporation adequate throughout the experiment for cooling him at this metabolic rate. S. R. walked

at 5.6 km./hr. up a 2.5 per cent grade (total M.R. 196 Cal./m<sup>2</sup>/hr.) and maintained constant rectal and skin temperatures of about 38.2°C. and 36.8°C. respectively, from the 2nd through the 4th hours of the exposure and then underwent a pronounced secondary rise of temperature during the 5th and 6th hours. The failure of S. R. to continue in thermal equilibrium through the last 2 hours apparently lay in his inability to maintain during the 5th and 6th hours a rate of evaporation high enough to dissipate all of the heat of metabolism plus the heat

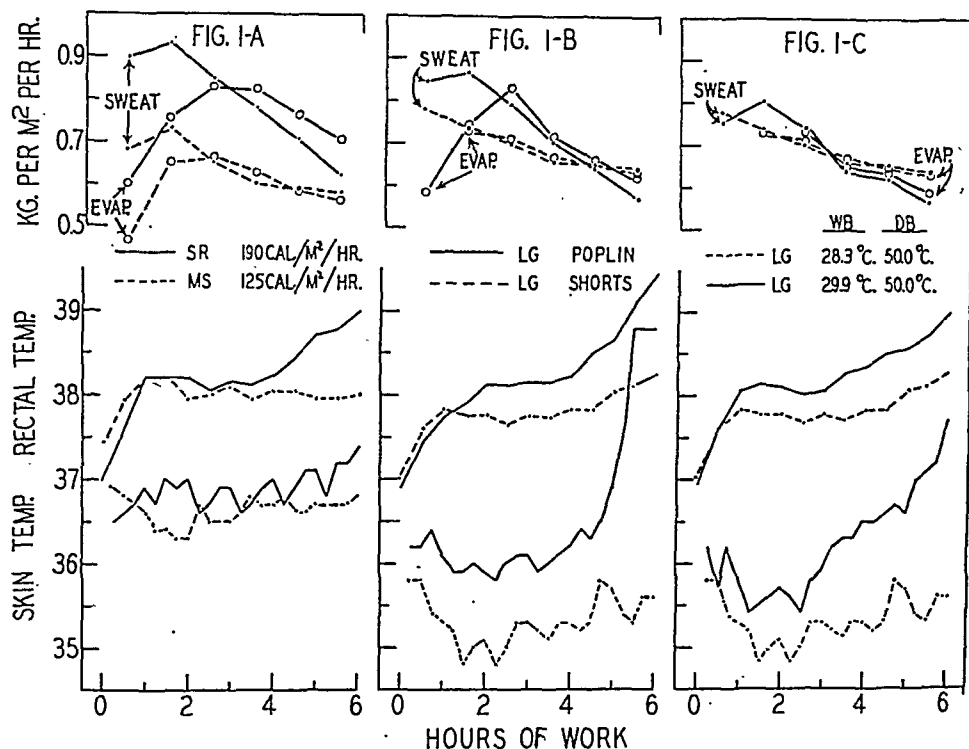


Fig. 1-A. Effects of two intensities of work on men clothed in poplin tropical suits. The dry bulb temperature was 50.0°C, and the wet bulb temperature was 28.0 in both experiments.

Fig. 1-B. Effects of a poplin tropical suit and of shorts on the thermal balance of a man walking at 5.6 km./hr. up a 2.5 per cent grade. The dry bulb temperature was 50.0°C. and the wet bulb temperature was 28.3 in both experiments.

Fig. 1-C. Effects of wet bulb temperatures of 28.3 and 29.9°C. at a constant dry bulb temperature of 50.0°C. on the thermal balance of a man walking at 5.6 km./hr. on a 2.5 per cent grade. The subject wore shorts in both experiments.

absorbed from the environment. The failure to keep evaporation high enough to dissipate all of his heat was due to a steady decline in his sweating rate after the 2nd hour which resulted in a decrease in his evaporation during the last half of the experiment. During the first three hours of the experiment sweating was higher than evaporation and his clothing accumulated a total of 824 grams of sweat. During the last 3 hours his sweating rate had declined to such an extent that one-third of the moisture in his clothing was dried out by evaporation.

Using the method of partitional calorimetry described by Winslow, Herrington and Gagge (1936) and Gagge (1936) we have partitioned the average heat exchange of S.R. and M.S. for the 2nd, 3rd and 4th hours of the above experiments, periods during which both men were virtually in thermal equilibrium (table 2). Since we did not measure surface temperature of the men's clothing, the values of radiation plus convection were taken as the remainder after the heat of metabolism was subtracted from the sum of evaporative heat loss plus any gain of stored body heat during the period. Changes of stored body heat were calculated according to Burton (1935) assuming 0.83 as the average specific heat of the body. There was a difference of 93 Cal./m<sup>2</sup>/hr. in the heat of vaporization of moisture from the two men as compared with a difference of only 59 Cal./m<sup>2</sup>/hr.

TABLE 2

*Partitional heat exchange of subjects S. R. and M. S. working at different metabolic rates*

The men were clothed in poplin suits and the air temperature was 50°C. with a wet bulb temperature of 28°C. in each experiment. All values are expressed in Cal. per meter square of body surface per hour.

SUBJ.	HEAT OF METAB.*	EVAP.	HEAT STORED	RAD. PLUS CONV.†
Av. during 2nd, 3rd and 4th hrs.				
S. R.	184	463	0	279
M. S.	125	376	-1	250
Diff.....	59	93	1	29
Av. during 5th and 6th hrs.				
S. R.	198	421	11	234
M. S.	125	330	0	205
Diff.....	73	91	11	29

\* Heat of metabolism is the total metabolism of the man minus the work done in walking up the grade.

† Radiation plus convection is the remainder after the heat of metabolism was subtracted from the sum of evaporative heat loss plus gain of stored body heat.

in metabolic heat production. Thus from the data it appears that S. R., working at the higher rate, gained 29 Cal./m<sup>2</sup>/hr. more heat by radiation and convection than did M.S. This difference was due in part to the effect of increased speed of bodily movement on convective heat exchange. Another factor which may have contributed to the difference in heat gain was the fact that the clothing of S.R. was kept wetter with sweat than that of M.S. and thus probably had a lower surface temperature during the equilibrium period; clothes of S.R. contained a total of 824 grams of sweat and those of M.S. 547 grams. Since his clothing was wetter, a greater fraction of his sweat evaporated from his clothing and may have been less efficient in cooling the body than evaporation directly from the skin. Similar data on the men's heat exchange in the 5th and 6th hours of the ex-

periments are also given in table 2. In both men evaporation and heat gain by radiation and convection during this period decreased significantly below values observed on them during the preceding equilibrium periods. S.R., continuing the harder work, showed during the 5th and 6th hours a moderate rise of metabolism and accumulated 11 Cal./m<sup>2</sup>/hr. of body heat, whereas M.S. continued the lighter work and remained in thermal equilibrium in spite of having his evaporation decline 46 Cal./m<sup>2</sup>/hr. Since environmental conditions were the same and remained constant throughout both of these experiments this decline in heat gain from the environment by the men is not explainable on the basis of available data. It was accompanied by the partial drying of the clothing of both men.

Figure 1-B shows the effects of clothing worn by subject L.G. during 6-hour walks at the standard rate, i.e., 5.6 km./hr. up a 2.5 per cent grade. The air temperature was 50°C. with a wet bulb temperature of 28.3°C. in both experiments. Under these conditions L. G. exhibited much less evidence of strain when he wore only shorts, shoes and socks than when he was clothed in a poplin tropical suit. In the latter clothing he was under the same stress as described for subject S. R. in the preceding paragraph and he showed the same type of failure to maintain thermal equilibrium. His skin and rectal temperatures were distinctly lower and the characteristic decline in sweating less in the exposure in which he wore shorts. The total amount of sweat he secreted during the first 4 hours was 700 cc. more in clothing than in shorts, whereas during the last 2 hours his sweating and evaporation declined to lower levels when he wore clothing than when he wore shorts. Associated with this failure in evaporation he showed a rise of 5 per cent in metabolism and a particularly pronounced rise of body temperature near the end of the clothing experiment. Table 3 gives the partitioned heat exchange of subject L. G. at four stages of these two experiments. Although the work performed was the same in the two experiments his heat of metabolism was from 5 to 10 per cent higher during the clothing experiment. This was undoubtedly due in part to the difference in body temperature in the two experiments. During the first hour of the experiment in which he wore shorts evaporation could not be determined because an unmeasured amount of sweat dripped from him. During the 2nd hour of the clothing experiment, before he had attained thermal equilibrium, his evaporation and heat gain by radiation plus convection were both about the same as corresponding values in the experiment in which he wore shorts. Gagge, Winslow and Herrington (1938) found in resting men exposed to operative temperatures of 36 to 40°C. that heat gain by radiation plus convection was greater when the men's skin was bare than when they were clothed. During the 3rd and 4th hours of both experiments our subject maintained thermal equilibrium, but his heat of vaporization of moisture was increased to 442 Cal./m<sup>2</sup>/hr. in clothing and declined to 394 Cal./m<sup>2</sup>/hr. for the same period in shorts. A difference of 10 Cal./m<sup>2</sup>/hr. in his heat of metabolism during this period accounts for only one-fifth of the difference in evaporative requirement between the two experiments, the other four-fifths being due to increased absorption of heat by radiation and convection. This difference is

opposite to the difference observed by Gagge et al. (1938) during 150-minute exposures of resting men to milder heat stresses. Our subject's increase of heat gain from the environment during the period of thermal equilibrium was probably related to the fact that his clothing had become about half saturated with sweat and this involved increased evaporation from the fabric, lowering its surface temperature. It will be noted that in both experiments he showed a decrease in heat gain by radiation and convection between the equilibrium periods and the later hours of the experiments similar to the decreases observed on subjects

TABLE 3

*The effects of the poplin tropical suit and of shorts on the partitional heat exchange of L. G. walking at 5.6 km./hr. up a 2.5 per cent grade*

The dry bulb temperature was 50°C. and the wet bulb temperature 28.3°C. in both experiments. All values are expressed in Cal. per meter square of body surface per hour.

CLOTHING	HEAT OF METAB.*	EVAP.	HEAT STORED	RAD. PLUS CONV.†
2nd hr.				
Poplin.....	180	420	6	246
Shorts.....	173	417	0	244
Diff.....	7	3	6	2
Av. 3rd and 4th hrs.				
Poplin.....	183	442	3	262
Shorts.....	173	394	2	223
Diff.....	10	48	1	39
Av. 5th and 6th hrs.				
Poplin.....	192	354	26	188
Shorts.....	178	360	5	187
Diff.....	14	-6	21	1

\* Heat of metabolism is the man's total metabolism minus the work done in walking up the grade.

† Radiation plus convection is the remainder after the heat of metabolism was subtracted from the sum of evaporation plus gain of stored body heat.

M. S. and S. R. This was associated with the corresponding declines of sweating and evaporation in both experiments and a partial drying out of the garments in the clothing experiment. It is clear from these data that studies of the heat exchange of working men involving only one or two hours of exposure are apt to be misleading.

Figure 1-C shows the effect on the heat exchange of subject L. G. of raising the wet bulb temperature from 28.3 to 29.9°C. with a constant dry bulb temperature of 50°C. in both experiments. The subject was exposed to each of these en-

vironments in separate experiments during each of which he walked for 6 hours at the standard rate. In the experiment in which the wet bulb temperature was  $29.9^{\circ}\text{C}$ ., his skin and rectal temperatures were higher and the total amount of sweat secreted during the first 3 hours was greater than at the lower wet bulb temperature. Associated with his high temperature and rate of sweating in the first half of the experiment at the higher wet bulb temperature was a decline to a considerably lower sweating rate during the last 2 hours and this reduced evaporation and caused a further pronounced rise in both skin and rectal temperatures.

Data in figures 1-A, 1-B and 1-C on men working at rates near the limits of their ability to maintain thermal equilibrium in an air temperature of  $50^{\circ}\text{C}$ . show that increases of stress, whether produced by increasing the work intensity, the clothing or the wet bulb air temperature, other factors remaining constant in each case, all caused the same type of failure of the men to regulate body temperature. The failure was associated with a gradual decline during the last two-thirds of the experiments in the men's sweating rates resulting in inadequate evaporative cooling during the 5th and 6th hours. There was also a moderate elevation of metabolic rate associated with the elevated body temperatures at the end of the experiments. The decline of sweating occurred in all of the experiments carried out under the above conditions of work and environment, its amount and importance being increased by increasing the stress.

Figure 2-A shows a comparison of the effects of dry and humid heat on the rates of sweating and body temperatures of men working at the standard rate. These experiments were carried out under the most severe heat stress in which the men could continue in thermal equilibrium from the second through the sixth hours of exposure. It will be noted that the skin and rectal temperatures of the men were distinctly higher in humid heat than in dry heat. Despite this difference in body temperature the men's average rates of sweating during the first two hours of exposure were about the same in the two environments. In addition to having higher body temperatures in humid heat the men underwent much greater declines in their rates of sweating during the last 4 hours of the exposures than they did in dry heat. Since an unmeasured amount of sweat dripped from the men in humid environments, it is impossible to calculate the partitioned heat exchange of the men in these experiments.

Figure 2-B gives a comparison of the effects of dry and humid environments on men when the heat stress in each case was just severe enough to cause a definite failure of the men to maintain thermal equilibrium and yet allow them to complete 6 hours of work at the standard rate. The environmental conditions during these experiments are described in connection with figure 2-B. It will be noted in the data that the rise in body temperature which occurred during these breakdown experiments followed a distinctly different course in humid heat from that observed in dry heat. During the second, third and fourth hours of the exposures the men were considerably hotter in the humid than in the dry environment. Excess sweat dripped from the men throughout the experiments in humid heat indicating that environmental resistance to evaporation was the

limiting factor in cooling and because of this the men were not able to remain in thermal equilibrium for any considerable period of time, even during the time when sweating was at its highest rate. As described above, the experiments in dry heat were characterized by a long period during which the men were in thermal equilibrium and after which they experienced a rapid rise of

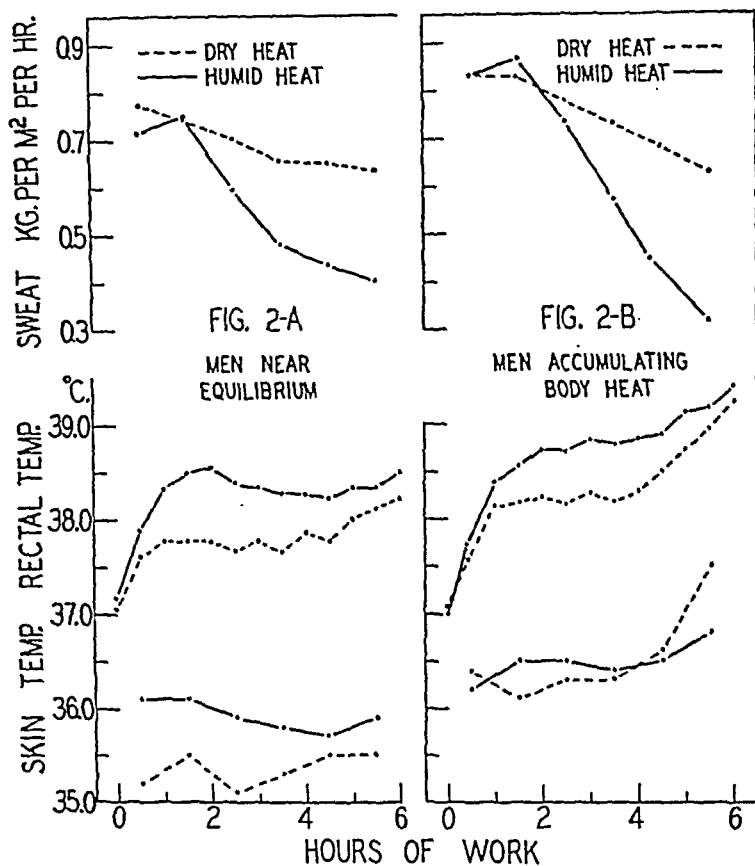


Fig. 2-A. The effects of dry heat (dry bulb 50°C. with a wet bulb of 28.3°C.) on subject L. G. wearing shorts as compared with the effects of humid heat (dry bulb 32.6°C. with a wet bulb of 31.6°C.) on L. G., S. R. and M. S. wearing the poplin tropical uniform. The values are averages on 3 men. The men walked on the treadmill at 5.6 km./hr. up a 2.5 per cent grade in all experiments.

Fig. 2-B. The effects of dry heat (dry bulb 50°C. with a wet bulb of 28.3) and of humid heat (dry bulb 33°C. with a wet bulb of 32.0) on the responses of subjects L. G., S. R. and M. S. All values plotted are averages for the three subjects wearing the poplin tropical suit and walking at 5.6 km./hr. up a 2.5 per cent grade.

temperature during the last two hours. During the equilibrium period evaporative cooling was adequate and the rise in temperature at the end was associated with a physiological limitation of evaporation, i.e., with a decline in sweating. The men's initial rates of sweating were the same in the two environments but the decline in sweating during the last four hours was much greater in humid than in dry heat. Since the men's sweating was always in excess of evaporation

in humid heat there was physiological economy in the rapid reduction of sweating which occurred in this type of environment. It should be emphasized that the

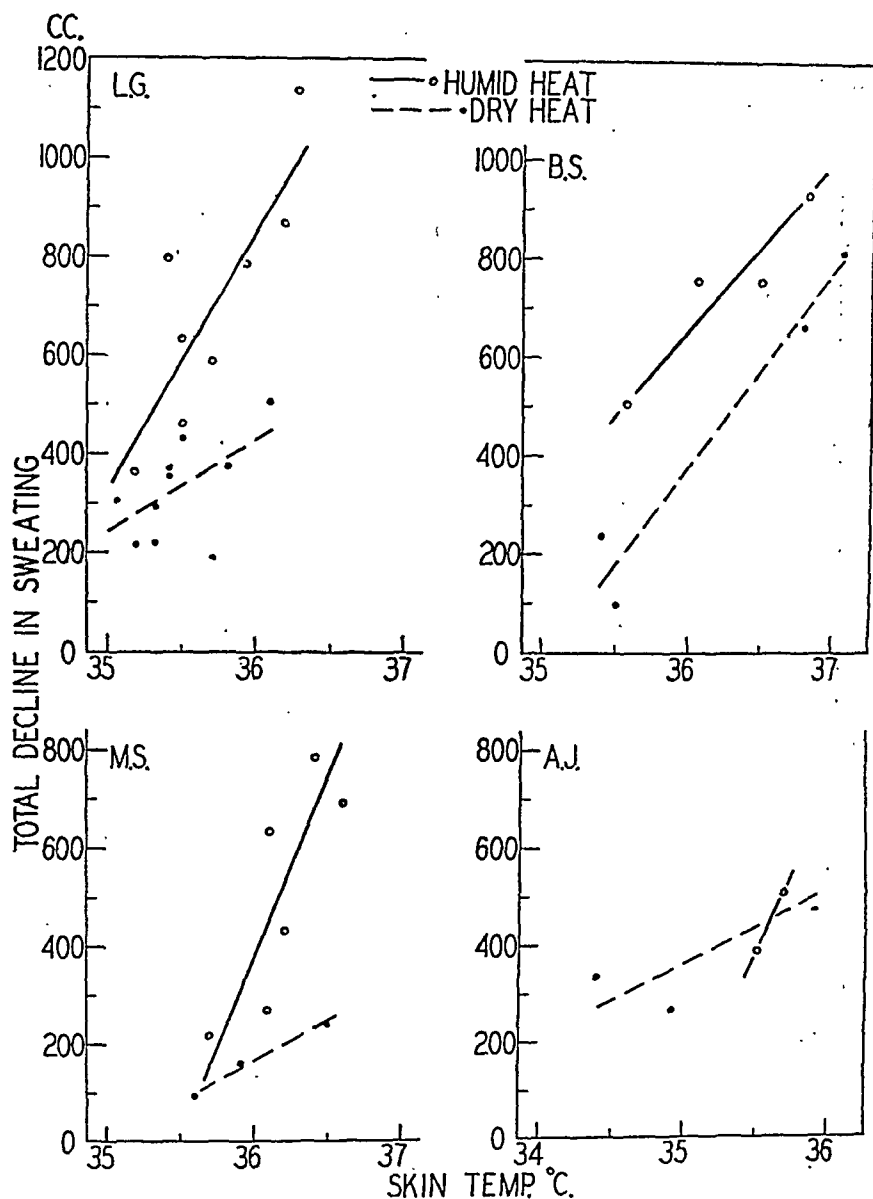


Fig. 3. The average skin temperature in relation to the decline of sweating of men walking at 5.6 km./hr. up a 2.5 per cent grade during 40 separate 6-hour exposures to severe heat. The variations of skin temperature and sweating between the different experiments on each subject were produced by varying atmospheric temperatures ( $^{\circ}\text{C}.$ ) within the following ranges:

	Mildest		Most severe	
	Wet bulb	Dry bulb	Wet bulb	Dry bulb
Humid heat.....	32.0	31.0	34.0	32.6
Dry heat.....	40.5	27.0	50.0	30.0

greater decline in sweating in this comparison was associated with higher skin and rectal temperatures.

The men could sweat at constant rates for six hours without exhausting their ability to sweat when their skin was not warmer than  $34.5^{\circ}\text{C}$ . and the evaporative requirement for thermal balance did not exceed  $0.4 \text{ kgm./m}^2\text{/hr}$ . This is shown by experiments on four men wearing shorts and walking at the higher rate in moderate heat (dry bulb temperature  $38^{\circ}\text{C}$ . and wet bulb  $24.5^{\circ}\text{C}$ .). From the second through the sixth hours of the exposures they maintained practically constant sweating rates averaging  $0.4 \text{ kgm./m}^2\text{/hr}$ . and constant skin and rectal temperatures averaging  $34.5$  and  $37.8^{\circ}\text{C}$ . respectively. They then continued the work for a 7th hour under greatly increased heat stress (dry bulb  $41.4^{\circ}\text{C}$ ., wet bulb  $33.4^{\circ}$ ); during this period their rectal and skin temperatures rose to averages of  $38.9$  and  $37.1^{\circ}\text{C}$ . respectively and they were able to increase their average sweating rate to  $0.67 \text{ kgm./m}^2\text{/hr}$ . This showed that the 6-hour exposure of the men to moderate heat stress did not cause the exhaustion of the sweating mechanism which characteristically occurred in severe exposures in which sweating continued to decline through 6 hours despite rising body temperature.

Figure 3 shows the relation between the decline of sweating and the skin temperature of men working in extreme heat. This figure gives the data from forty 6-hour experiments on four subjects walking at  $5.6 \text{ km./hr}$ . up a 2.5 per cent grade with heat stresses near the limits of their ability to maintain thermal equilibrium. Twenty of these experiments were carried out in dry heat and 20 in humid heat. The men's total rates of sweating declined in all of these experiments, the amount of the decline in each man tending to be greater the higher his average skin temperature during the exposure. The differences in skin temperature in these experiments were due to varying heat stress; i.e., the greater the heat stress the higher the subject's skin temperature. It is evident from the data that the relationship was different in dry heat and humid heat, i.e., the decline was not as great for a given skin temperature when a man was working in dry heat as in humid heat. The data also show individual variations in the decline of sweating among the four men.

Data given in figure 4 indicate that the skin temperature locally affects the rate of sweating. Six-hour experiments were carried out on three men wearing shorts and working at the higher rate in humid heat (dry bulb temperature,  $34.5^{\circ}\text{C}$ .; wet bulb temperature,  $33.5^{\circ}\text{C}$ .). The average skin temperature of the men varied only slightly and their average rectal temperature increased from  $38.2$  to  $38.5^{\circ}\text{C}$ . between the 2nd and 6th hours. Sweat samples were collected during each experiment in elbow length rubber gloves. One of each man's gloved hands was submerged in cold water and its skin temperature kept at an average of  $30.1^{\circ}\text{C}$ . The skin temperature of the other gloved hand was maintained at an average of  $36.9^{\circ}\text{C}$ . Because of the overall cooling effect through the cooled hand in these experiments a greater heat stress was necessary to tax the men's tolerance for 6 hours than would otherwise have been required. All of the men showed the characteristic decline in the total rate of sweating. The rates of sweating of both hands declined steadily during the last four hours of the experiments but during the same period the cool hand sweated from

48 to 96 per cent more than the warm hand. The percentage decline in the men's rates of sweating from the average of the first two hours to the 6th hour was 59 for their cool hands in which skin temperature was maintained at  $30.1^{\circ}\text{C}.$ , 68 for the total skin surface where the average temperature was  $35.8^{\circ}\text{C}.$  and 76 for the warm hands whose surface temperature averaged  $36.9^{\circ}\text{C}.$  The fact that the locally cooled hand maintained the higher rate of sweating indicates an effect of the heat in depressing sweating in the other hand which was, at least in part, direct and not entirely through the central nervous system. The decline in sweating which occurred in the cool hand could have involved direct

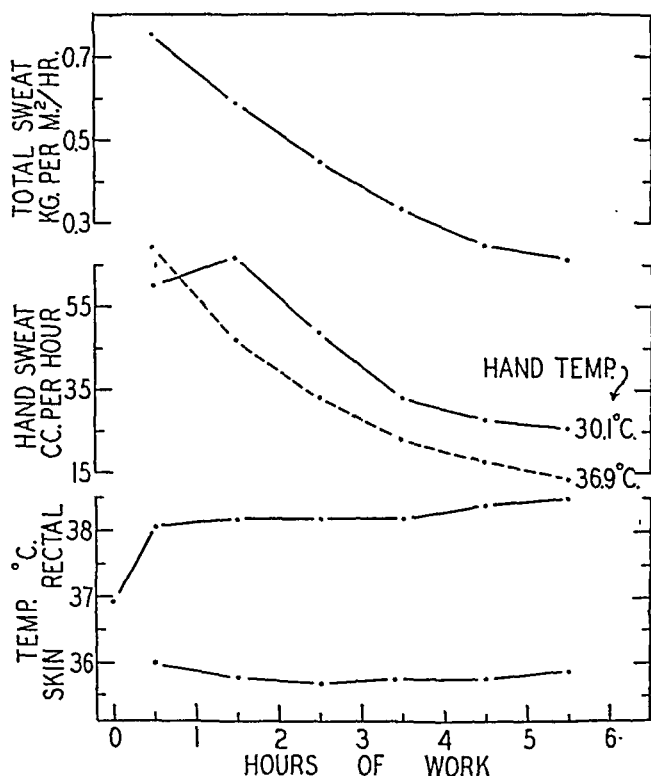


Fig. 4. The decline of sweating as shown by simultaneous determinations on: (a) the general body surface (average skin temperature  $35.8^{\circ}\text{C}.$ ), (b) a hand maintained at a surface temperature of  $30.1^{\circ}\text{C}.$ , (c) the other hand, temperature  $36.9^{\circ}\text{C}.$  The values are averages on three men.

fatigue of the sweat glands brought on by the prolonged high rate of sweating. Fatigue of the sweating reflex associated with the long continued high temperature of the general skin surface and a central effect of the high internal body temperature are other factors which may have contributed to the decline of sweating in the cool hand.

DISCUSSION. In these 6-hour experiments the relations of the men's changes in body temperature to clothing and metabolic rate and to environmental or physiological limitations of evaporation are obvious from the results given above. Further investigations are necessary to explain the effects of clothing

and of variations in its moisture content in increasing the amount of evaporation required for equilibrium by the men working in dry heat. The increases of the men's metabolic rates which occurred during the course of the more severe exposures probably were related to their rising body temperatures and progressively developing fatigue. DuBois (1921) gave proof that a rise of body temperature in resting men causes an increase in metabolism according to Vant Hoff's Law.

We do not know the fundamental cause of the decline in sweating shown by the men in these prolonged exposures to extreme heat and therefore must be content with an enumeration of the conditions under which it occurred. In all of the 6-hour experiments in which men have shown a significant decline in their sweating rates the following physiological conditions have prevailed. (1) The men were previously well acclimatized and trained for prolonged work in severe heat. (2) They were fully hydrated with 0.1 per cent salt solution throughout the exposure. (In other experiments we have found that men's rates of sweating declined in a similar manner when they abstained from drinking water or when they drank unsalted water ad lib during periods of 3.5 to 6 hours of work in the heat.) (3) The decrease in sweating was not caused by a fall in body temperature because it occurred regularly during periods when the men's skin and rectal temperatures were constant or rising. (4) After the first hour of experiments in which the men underwent a decline in sweating their skin temperatures were always above  $35^{\circ}\text{C}$ ., their rectal temperatures above  $37.8^{\circ}\text{C}$ . and their initial rates of sweating above  $0.65 \text{ kgm./m}^2\text{/hr}$ . (5) In general the amount of decline in sweating by a given man increased with increments in his skin temperature, rectal temperature and initial sweating rate. In the range of conditions used in these experiments these functions were raised by increasing heat stress. Gerking and Robinson (1946) presented data showing a significant positive correlation between the average rate of sweating in the first two hours and the amount of the decline of sweating occurring in men during the following 4 hours of work in severe heat. (6) For a given skin temperature or initial rate of sweating the decline in sweating was significantly greater in humid than in dry heat.

The decline of sweating of the men in these prolonged exposures is a possible factor in a gradual onset of the more complete failure of heat regulation which occurs in heat stroke. In the more severe exposures heat stroke would undoubtedly have occurred soon had the men been forced to continue working beyond 6 hours. It should be emphasized that the pronounced reduction in sweating by men working in humid heat when evaporation is reduced by the environment may be considered a useful adaptation in conserving body water. In case of water shortage in desert heat the reduction in sweating would assure an economical use of available water. This type of failure of men working in hot environments is undoubtedly important in certain heavy industries such as the steel mills and deep mines and also in prolonged infantry operations in extremely hot climates. Further studies should be made to determine the time

required for complete recovery from severe exposures and the cumulative effects of repeated exposures. A careful study should be made to determine the degree of strain shown by men in standard industrial situations.

#### SUMMARY

In dry heat (air temperature  $50^{\circ}\text{C}$ . with wet bulb temperature  $28.3^{\circ}\text{C}$ .) a man, clad only in shorts, shoes and socks, and walking on the treadmill with a total metabolic rate of  $185 \text{ Cal./m}^2\text{/hr.}$ , was virtually in thermal equilibrium with a rectal temperature of about  $38^{\circ}\text{C}$ . and a skin temperature of  $35.4^{\circ}\text{C}$ . from the 2nd through the 6th hours of the exposure. When fully clothed in tropical suits in the same work and environment men attained thermal equilibrium during the 3rd and 4th hours and then experienced a pronounced secondary rise in both skin and rectal temperature during the 5th and 6th hours. The rise in body temperature occurring in the last two hours was always associated with decreased evaporation because of a decline in sweating and with a rise of metabolic rate above that of the equilibrium period. The decrease in sweating occurred gradually during the last 4 or 5 hours. After the clothing had accumulated 400 to 800 grams of sweat during each experiment it increased the men's heat gain by radiation and convection to values well above any observed when the men wore shorts. In the same environment, reducing the work so that the metabolic rate was  $125 \text{ Cal./m}^2\text{/hr.}$  made it possible for the clothed men to continue in thermal equilibrium through the 6th hour.

The most severe humid heat in which clothed men with metabolic rates of  $190 \text{ Cal./m}^2\text{/hr.}$  could maintain thermal equilibrium in the 6-hour experiments was an air temperature of  $32.6^{\circ}\text{C}$ . with a wet bulb of  $31.6^{\circ}\text{C}$ . These equilibrium experiments were characterized by greater declines in the men's sweating rates and by skin and rectal temperatures which were both about  $0.5^{\circ}\text{C}$ . higher than occurred in the most severe equilibrium experiments in dry heat.

The fatigue of the sweating mechanism of the men in these 6-hour experiments always occurred when their skin temperatures were maintained constant at high levels ( $35$  to  $37^{\circ}\text{C}$ .) for long periods and when their initial rates of sweating in the exposures were high ( $0.65 \text{ kgm./m}^2\text{/hr.}$  or more), the amount of the decline in sweating being positively correlated with both of these measurements.

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# THE EFFECT OF INSULIN UPON THE TOLERANCE OF THE EVISCERATED RAT FOR INTRAVENOUSLY ADMINISTERED GLUCOSE

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This is one of a series of studies on the metabolic effects of hormones in the eviscerated rat. Intravenous infusions of saline and of different concentrations of glucose and insulin were made for periods of 2 hours and 24 hours in an effort to determine the dose-response relationship between insulin concentration and glucose tolerance and the range in variability of response among individual animals. It was demonstrated that insulin has a striking effect upon the ability of the eviscerated rat to remove glucose from its blood and that the glucose tolerance gradually decreases during the period of survival. The variability among individual animals was large.

**METHODS.** Twelve hundred male rats of the Sprague-Dawley strain were used in these experiments. The diet was Purina Dog Chow. When the rats reached a weight of 185 to 205 grams, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of  $250 \pm 2$  grams, they were anesthetized (intraperitoneal injection of 18 mgm. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure described by Ingle and Griffith (1). All of the intra-abdominal organs were removed except the adrenals and kidneys.

Intravenous infusions of a 0.9 per cent sodium chloride solution with and without added glucose and crystalline zinc insulin (Lilly) were made by two continuous injection machines which delivered fluid from each syringe at the rate of 20 cc. in 24 hours. Syringes of the Luer-Lok type were selected to deliver 20 cc. with a stroke of 65 mm. Two syringes were operated by one machine and six syringes by the second machine. Each machine was powered by a synchronous motor, and the reduction of motion was achieved by a precision-built system of gears so that an exact control of the rate of injection was maintained.

The infusions were made into the saphenous vein of the right hind leg and were started within five minutes following the removal of the liver. The animals were secured in a supine position on an animal board. In these liverless animals one initial dose of the barbiturate was sufficient to maintain effective anesthesia throughout the experiment. The temperature of the room was maintained at 75 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation. It is possible that a more precise control of temperature would reduce the variability in results among individual animals.

The analyses of blood glucose were made by the method of Miller and Van Slyke (2). This procedure measures small amounts of non-fermentable reducing

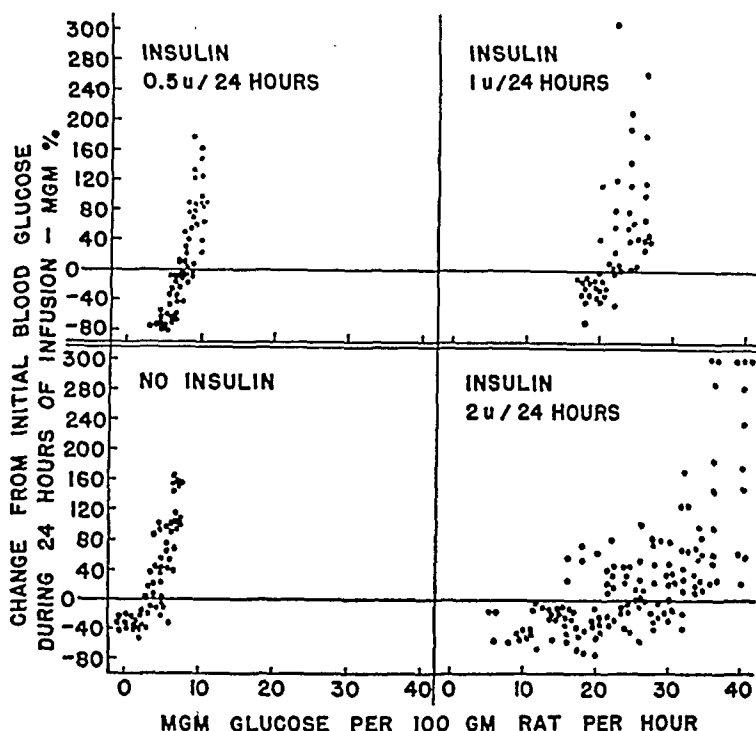


Fig. 1.

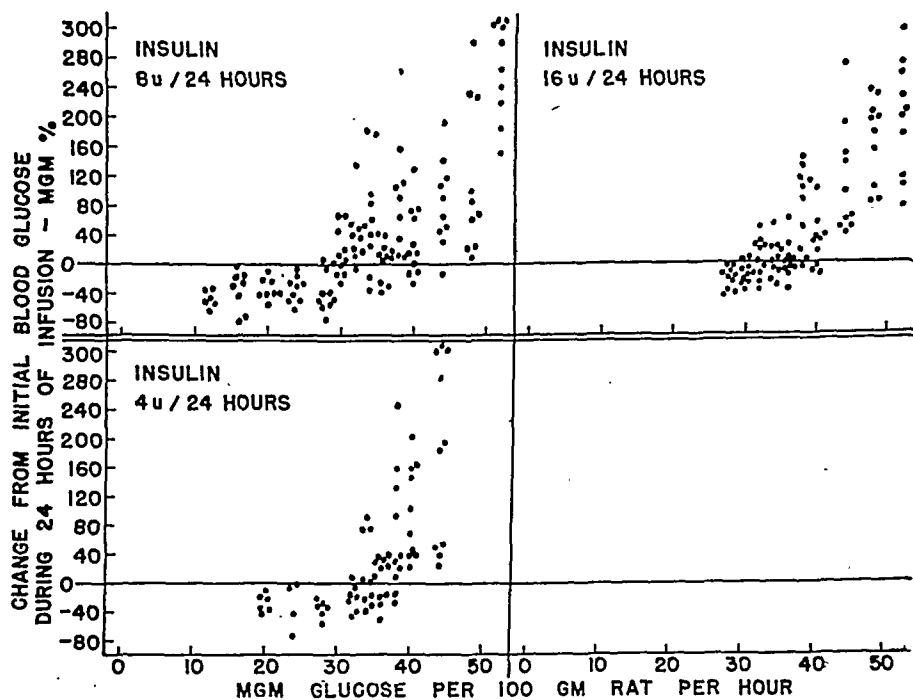


Fig. 2.

Fig. 1 and 2. The effect of insulin on the tolerance of eviscerated rats for glucose during 24 hours. Ten rats were tested at each level of glucose load. At low levels of glucose intake many rats died of hypoglycemia. Among the rats given insulin all died when the glucose load was less than 8/100/h. Results are shown for surviving animals only.

substances which accumulate in the blood of eviscerated rats. Samples of blood were taken from the tail just prior to the beginning of infusions and after two hours of infusion. At the end of 24 hours the sample of blood was taken from the jugular vein.

**EXPERIMENTS AND RESULTS.** In experiment 1 (figs. 1 and 2) the tolerance to glucose was determined with and without insulin over a 24-hour period. The levels of insulin dosage were 0.0, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 units per rat per 24 hours. Different loads of glucose were used at each level of insulin dosage. Ten rats were tested at each concentration of glucose and insulin. The amount of change in the level of blood glucose during the 24 hours was the index of tolerance. At all levels of insulin intake the glucose load was changed from insufficient amounts which permitted the blood glucose to decrease in all

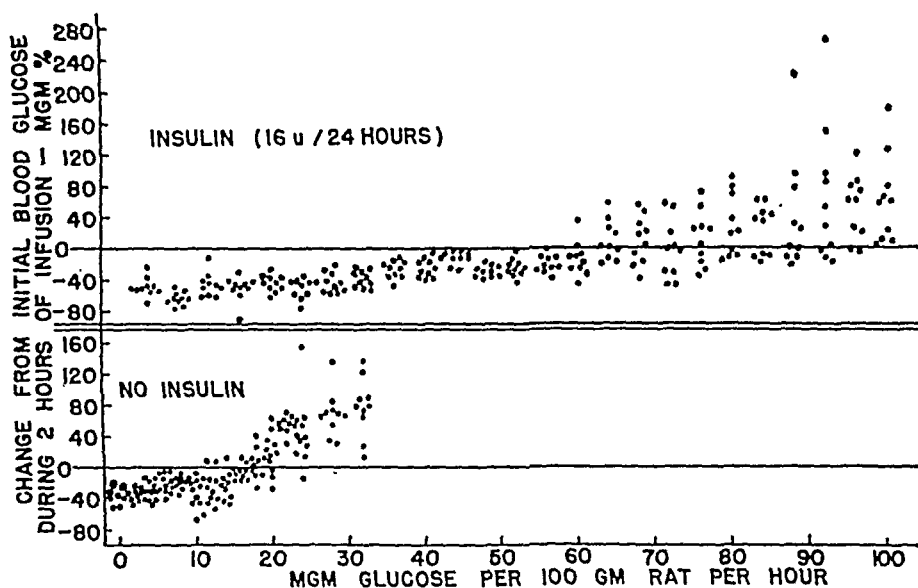


Fig. 3. The effect of insulin on the tolerance of eviscerated rats for glucose during 2 hours. Ten rats given insulin without glucose died. All of the animals given glucose survived.

ten animals to excessive amounts which caused a rise in blood glucose in all ten of the test animals. The results are expressed as milligrams of glucose per 100 grams of rat per hour (mgm./100/h.). There is no exact relationship between body-weight and glucose utilization, but body-weight was constant in these experiments so that the expression is usable.

Experiment 2 (fig. 3) was identical with experiment 1, except that the tolerance to glucose was studied over a 2-hour period following evisceration, and animals without insulin were compared to animals given insulin at the rate of 16 units per rat per 24 hours.

As shown earlier by Ingle and Sheppard (3), the administration of insulin without glucose to the eviscerated rat shortens the time of survival. At optimal levels of insulin dosage some animals died with hypoglycemia within 2 hours.

The approximate average glucose load required to sustain the blood glucose for 24 hours at different levels of insulin dosage (expt. 1, figs. 1 and 2) are as follows: no insulin, 4/100/h.; 0.5 unit of insulin, 8/100/h.; 1 unit of insulin, 21/100/h.; 2 units of insulin, 28/100/h.; 4 units of insulin, 36/100/h.; 8 units of insulin, 36/100/h.; and 16 units of insulin, 36/100/h. Some of these values are somewhat arbitrary selections because of the great variability in response to increment in glucose load.

Within a period of two hours (expt. 2, fig. 3) the amounts of glucose tolerated per unit time were much greater. Without insulin the approximate average glucose load required to sustain the level in the blood was 17/100/h., and with a super-optimal amount of insulin 74/100/h. was required.

Although an increase in glucose load was usually followed by an average increase in level of blood glucose, the range in variability among individual animals was great. For example, among the animals studied for 2 hours without insulin there was overlapping in the responses of animals given no glucose with those of the series given 20/100/h., and in the same experiment the animals given super-optimal amounts of insulin showed overlapping in response between animals receiving 4/100/h. and animals receiving up to 76/100/h. In the accompanying charts (figs. 1, 2 and 3) considerable overlapping can be observed in both the hypoglycemic and hyperglycemic phases of response.

DISCUSSION. Soskin and Levine (4) have reviewed the studies on the hypoglycemic action of insulin in the liverless animal. In the present studies the differences in amounts of glucose "utilized" by eviscerated rats with and without insulin were very large. We do not know the manner of its utilization. From studies by Russell (5) it might be assumed that the glucose was oxidized rather than stored or converted. Relative to this assumption, it is of interest that larger amounts of glucose were tolerated with insulin at either hypoglycemic or hyperglycemic levels than at hyperglycemic levels in animals not treated with insulin. However, the effect of insulin upon the oxidation of the injected carbohydrate in this preparation cannot be satisfactorily appraised without full knowledge of changes in stores of fat and carbohydrate in the entire animal and the extent of gluconeogenesis in extra-hepatic tissues.

The ability of the eviscerated rat to remove glucose from its blood decreases during its period of survival. Animals which reach the end of a 24-hour test period with blood glucose at its initial level have passed through an early phase of hypoglycemia with gradual recovery. Possibly the insulin remaining in the blood at the time of evisceration has significant effects before it is exhausted, but this is not the major factor causing the difference in glucose tolerance at the 2-hour and the 24-hour periods. A marked difference persisted in the presence of super-optimal amounts of insulin. It is probably closely related to the progressive decrease in oxygen consumption noted by Roberts, Samuels and Reinecke (6). Can the change in tolerance be due to changes in the rate of gluconeogenesis by the kidney? Preliminary studies have shown that the eviscerated, nephrectomized rat also shows a striking response to insulin and a progressive decrease in glucose tolerance either with or without insulin.

The variability in the response of individual animals to a difference in glucose load has been noted. The responses were relatively uniform among animals tested at the same time, but cycles of change in response covering intervals of several days or weeks were noted. These were not due to experimental errors in the sampling or measuring of blood glucose or to differences in the preparation of the animals. It is considered that changes in temperature, although small, may have been a major factor in causing cyclic variability. Marked variability in glucose utilization has been noted by Houssay, Dosne and Foglia (7) in the liverless dog. On the basis of our experience we have concluded that in any comparative studies in the eviscerated rat the control and experimental animals should be tested simultaneously. Indeed, we have no experience with any kind of biologic testing in which this principle can profitably be ignored.

#### SUMMARY

Male rats were eviscerated at a weight of 250 grams and were given continuous intravenous infusions of saline with different concentrations of glucose and insulin in order to determine the dose-response relationship between insulin and glucose tolerance. Test periods of 2 hours and 24 hours were used. The approximate average glucose load required to sustain the blood glucose for 2 hours without insulin was 17 mgm. per 100 grams of rat per hour, and with an optimal amount of insulin (16 units per 24 hrs.) it was 74/100/h. Over a 24-hour period the values were: no insulin, 4/100/h., and with the most effective dose of insulin, 36/100/h. There was a marked variability among the responses of individual animals to a change in glucose load.

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# THE PRODUCTION AND EXCRETION OF UREA AND OXYGEN CONSUMPTION DURING INTESTINAL OBSTRUCTION IN RABBITS<sup>1</sup>

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One change of universal occurrence in intestinal obstruction is azotemia, the elevation of the blood non-protein nitrogen. A review (1) of the earlier literature indicates that most commonly two explanations have been offered for the azotemia. Some workers considered that it was due to an increased tissue catabolism brought about by toxins arising in the obstructed bowel. Their findings of an increased urinary nitrogen excretion in dogs with bowel obstruction are open to criticism. The surgical procedures, the sepsis, the contamination of the urine with vomitus in some cases and an insufficient data on the nitrogen excretion pre-operatively were complicating factors which make evaluation difficult. Other workers attributed the azotemia to impaired renal function which resulted from the dehydration.

Schnohr (2) concluded from a very extensive study of intestinal obstruction in the rat that part of the azotemia was due to the kidneys failing to excrete urea when the available chlorine was below a certain value. Furthermore, he concluded that part of the azotemia remained in spite of saline injection and that it showed no relation to the total chloride content of the animal, the serum or urine chlorine or the urine flow and therefore must be due to an increased formation of urea which could not be excreted as quickly as it was produced.

Gömöri and St. Freweisz (3) found that administration of 0.1 gram of NaCl per kilogram of body weight lengthened the life of cats with pyloric obstruction to a small degree. Treatment with 0.9 per cent saline resulted in life 2.5 times that of the cats receiving the hypertonic saline. The former did not prevent azotemia while the latter either prevented it or greatly delayed it. They concluded that the azotemia was due to the dehydration and not to the hypochloremia.

Gömöri and Podhradsizky (4) studied protein catabolism in cats with pyloric obstruction by determining the urinary non-protein nitrogen and the increase in the nitrogen content of the trichloroacetic acid filtrate of blood and tissues. The sum of these two values for obstructed cats was decidedly greater than that obtained for cats which had been deprived of food and water for 5 days. Therefore, they concluded that in obstruction there was an excess destruction of tissues which was not due to hypochloremia because administration of NaCl did not prevent it. In their tables, these workers report both the water and the non-protein nitrogen content of the blood and tissues. Using their data to calculate the concentration of the non-protein nitrogen in the tissue water, the values for muscle ranged from 632 to 1002 mgm. for 100 ml. of water, for liver 414 to 880

<sup>1</sup> Supported in part by a grant from the Wisconsin Alumni Research Foundation.

and for blood serum 140 to 150. Obviously they are including in their tissue analyses nitrogen compounds, not usually considered as non-protein nitrogen in the blood plasma.

The azotemia of intestinal obstruction might be due, as the literature has repeatedly suggested, to a failure in excretion and it might be due to an increased rate of production of urea and other nitrogenous substances in association with the toxemia. This paper reports a study of these two factors in rabbits with intestinal obstruction.

**EXPERIMENTAL.** The rate of urea excretion was determined in normal male rabbits for 16 to 20 hours after removal from food. Since the stomachs of these animals always contain much food no attempt was made to secure a postabsorptive state. Before the collection period the urinary bladder was catheterized

TABLE 1  
*Effect of obstruction upon urea production and excretion*

NORMAL					OBSTRUCTION				
Rabbit no.	Wt.	Serum urea	Estimated* body water	Urea excretion	Urea production	Serum urea	Body† water	Gain in body urea	Urea excretion
	kgm.	mgm./100 cc.	cc.	gms./hr.	gms./hr.	mgm./100 cc.	cc.	gms.	gm./hr.
1	1.62	44.1	1118	0.0761	0.0823	129.0	911	0.6822	0.0311
2	2.05	45.0	1415	0.0841	0.0835	149.2	1124	1.0403	0.0272
3	2.06	39.7	1421	0.0618	0.1442	284.2	1120	2.6189	0.0204
4	2.24	42.0	1546	0.0589	0.1210	237.0	1258	2.3321	0.0116
5	1.54	51.7	1063	0.1437	0.1659	147.0	893	0.7631	0.0987
6	1.90	37.5	1311	0.1136	0.1072	144.7	1097	1.0957	0.0136
7	3.46	41.1	2387	0.1837	0.1638	108.0	1941	1.1152	0.0812
8	2.68	39.0	1849	0.1620	0.1338	78.0	1460	0.4177	0.0946
9	2.46	87.1	1697	0.1165	0.0933	174.1	1301	0.787	0.0288

\* Calculated as 69 per cent of the body weight.

† Determined by desiccation at 105°C.

and washed with sterile saline. The same procedure was carried out at the end of the urine collection. The number of such determinations on each rabbit ranged from 7 to 15.

Under ether anesthesia, the intestine was ligated with a soft twine 15 cm. from the pylorus. In no animal at autopsy was there gross evidence of peritonitis except that in some there was hyperemia of the intestine adjacent to the ligature.

In order to determine the urea production during bowel obstruction it was necessary to know the total urea content of the animal. This was estimated by determining the concentration of urea in the blood serum and the product of this value and the total body water would give the total body urea. It is generally considered that urea is freely diffusible and its concentration in the cell water is identical with that of the extra-cellular fluid. Painter (5) has demonstrated that the total body water of dogs, determined as the volume of solvent for a known quantity of urea approximates that determined by complete desiccation. In the rabbits of table 1, the body water of the normal animal was estimated as 69 per

cent of its body weight, since this is approximate average obtained by complete desiccation of normal rabbits, and at post mortem after the obstruction it was determined by drying at 105° the entire animal after removal of the gastric and intestinal contents. In the rabbits of table 2, the body water was determined in the normal animal and near its terminus with bowel obstruction by the intravenous injection of a known amount of urea solution. The serum urea content was determined just before the injection and again about one hour later. The urea produced in this period was assumed to be at the same rate as had previously been determined for urea excretion during the first 4-5 hours after removal of the rabbit from food. The estimated quantity of urea produced in this period was added to the quantity injected. The urea excreted in this period was determined by catheterizing and washing the bladder. This amount was deducted from the sum of the quantities injected and produced. This remainder would give the

TABLE 2

*Effect of obstruction upon urea production and excretion. Body water determined as the volume available to dissolve a given amount of urea*

NORMAL					OBSTRUCTION				
Rabbit no.	Wt.	Serum urea	Body water	Urea excretion	Urea production	Serum urea	Body water	Gain in body urea	Urea excretion
	gm.	mgm./100 cc.	cc.	gms./hr.	gms./hr.	mgm./100 cc.	cc.	gms.	gm./hr.
A	4050	42.1	2543	0.1445	0.1698	100.4	1756	1.482	0.0442
B	2460	47.9	1499	0.1165	0.0872	106.3	1273	0.635	0.0329
C	4100	88.0	2506	0.1859	0.1271	119.6	2098	0.302	0.1296
D	3260	42.2	2496	0.1560	0.1027	113.3	1756	1.018	0.0385
E	2380	58.4	1618	0.1121	0.1648	97.2	1461	0.4757	0.1000
F	2890	84.1	2013	0.1370	0.0952	123.0	1566	0.2709	0.0782
G	3640	52.2	2360	0.1160	0.0402	67.7	1981	0.1112	0.0292
H	3820	54.0	2953	0.0998	0.0951	91.4	3047	1.1909	0.0218
RW	2980	55.4	2001	0.0998		101.9			0.0730

amount of urea available for solution, in the animal's body, which quantity divided by the increase in serum urea would give the quantity of water available to dissolve urea. The sum of the 1, increase in the total body urea; 2, the urea excreted during the intestinal obstruction, and 3, the urea in the contents of the obstructed bowel, when it was present, is considered as the amount of urea formed during the obstruction.

The oxygen consumption and carbon dioxide production was determined by a closed circuit similar to that described by Fredericia (6).

Urea was determined by urease and the manometric technique of Van Slyke (7). Serum urea was determined on the tungstate filtrate.

RESULTS. In tables 1 and 2 the marked increase in the serum urea during bowel obstruction is shown. It is seen in table 1 that in 4 rabbits of the 9, urea production during bowel obstruction exceeded the urea excretion of the normal rabbit. However, since the percentages of the increase in urea production were 8, 15, 105 and 133, it would seem that in only 2 of the 9 was urea production

significantly greater during obstruction than the normal. In table 2, urea production during obstruction exceeded the normal excretory rate in 2 of the 8 rabbits, the percentage increase being 17 and 47. Rabbit (RW) died before body water could be determined. The fluid in the obstructed bowel obtained by centrifugation amounted to 288 cc. which contained 0.1791 gram of urea. If the rabbit's urea formation during obstruction was that of its average normal urea excretion then 1.3802 grams would have been produced, of which 1.0074 grams were excreted. The remainder plus that in the obstructed contents, that is 0.5519 gram, would represent the urea which might dissolve in the body water. This volume might be considered to be the normal body water less the fluid of the obstructed contents. The urine volume would be negligible in this case. On this basis the serum urea should have risen 32 mgm. per 100 cc. but it actually rose 46.5 mgm., indicating that in this rabbit there was an increased rate of urea formation. Consequently, in table 2, three of nine rabbits had a higher urea formation during obstruction than when normal. Considering all of the 18 animals in tables 1 and 2, six had an increased rate of urea formation but of these only 3 seemed to have a significantly greater rate of urea formation.

The rate of urea excretion by the kidneys, with the exception of rabbits E and RW of table 2 was very much less than in the normal animal. Gamble and McIver (8) have pointed out that rabbits with pyloric obstruction are almost or entirely anuric. In 6 of the rabbits in this study during the first 7 hours of the obstruction, the urea excretion remained 90 per cent or more of their normal. In 4 others, after 3 to 5 hours of obstruction, the urea excretion was 65 to 70 per cent of their normal. Therefore, during bowel obstruction in rabbits there is a marked reduction in renal excretion. In some animals the impairment occurs during the last half of their course but in others it occurs during the early part of the obstruction.

As a control for the effect of ether rabbits 7 and 8 of table 1 and one other not tabulated, were subjected to ether anesthesia for a period of 8 to 25 minutes. Their urea excretion ceased during and for about an hour after the ether administration. This was followed by a diuresis, so that in periods of 2 to 5 hours after the anesthesia their urea excretion was approximately normal. In 2 other rabbits, the anesthesia and surgical procedure involved in producing bowel obstruction, with omission of the ligature, were carried out. Their urea excretion for 14 hours post-operatively was within their normal range. These control experiments suggest that other factors are responsible for the impairment in urea excretion which occurred in these experiments.

Schnedorf and Orr (9) have reported that distention of the entire small intestine in dogs anesthetized with pentobarbital, resulted in a decrease in bile flow and an increase of 11 to 81 per cent in urine flow. The effect of distention of Thiry fistula made in the first portion of the jejunum, upon simultaneous urea and creatinine clearances was studied in 3 normal, conscious dogs. This procedure was considered pertinent to the problem because Herrin and Meek (10) produced the picture of bowel obstruction by distention of such fistulae in dogs. A balloon was placed in the Thiry fistula of a trained dog and distended by an air pressure

of 90 mm. Hg or more. Normal clearance periods were made immediately preceding and following the 20 to 36 minute period of distention. In some experiments the distention was sufficient to induce vomiting. Twenty such periods of distention were carried out on the 3 dogs. In one dog the fistulous loop was continuously distended for 5 days, during which time the dog had vomited and seemed quite sick. Chronic distention seems to make the bowel more sensitive to distention (11). The balloon was then removed for a short time, saline administered and then the effect of distention upon the clearances was determined. However, in all of these distention experiments, there was no evidence that the urea and creatinine clearances were affected. The ratio of the two clearances to each other remained unchanged, indicating no increase in the tubular absorption

TABLE 3  
*Oxygen consumption and carbon dioxide production in intestinal obstruction*

RABBIT	CONDITION	PERIOD	O <sub>2</sub> USED PER MIN.	CO <sub>2</sub> PRODUCED PER MIN.	R.Q.
		<i>min.</i>	<i>cc.</i>	<i>cc.</i>	
6	Normal	103	19.55	17.77	0.81
	Normal	59	21.52	16.07	0.75
	Obstruction	58	10.53	12.38	1.18
	Obstruction	14	3.80	3.87	1.02
4	Normal	95	21.49	19.41	0.90
	Obstruction	56	11.25	12.17	1.08
	Obstruction	64	8.97	12.56	1.39
3	Normal	66	24.21	19.44	0.80
	Normal	88	22.75	20.23	0.89
	Obstruction	77	14.93	16.41	1.10
	Obstruction	63	15.30	15.18	0.99
5	Normal	115	19.79	14.60	0.73
	After ether	48	22.48	14.06	0.62
	Obstruction	64	7.64	8.56	1.12

of urea. Neither did the evidence indicate that intestinal distention had any effect on the rate of urine flow. These experiments seem to reject any idea that in dogs the azotemia of bowel obstruction could involve a reflex between the distended bowel and the renal function of excretion.

As seen in table 3, the oxygen utilization of rabbits in intestinal obstruction is very greatly reduced. In all of the animals used in this study, cyanosis of the lips was very marked during the last 2 to 3 hours of its life. This would be expected on the basis of the low respiratory intake of oxygen. Furthermore, all of the rabbits used in this study seemed to have a very little circulatory reserve. During the last 3 to 5 hours of their life, their ears were cold, pale and showed a limited and transient reactive hyperemia. When it was thought, on the basis of past observations, that their end was near, the removal of as little as 2 ml. of blood by heart puncture always precipitated the fatal termination.

**DISCUSSION.** The data of this study demonstrate that one factor responsible for the azotemia of intestinal obstruction in rabbits is insufficient renal excretion. It was the only factor in two-thirds of the animals.

The intestinal distention experiments with dogs previously described indicate that the renal failure is not likely on a reflex basis. McQuarrie and Whipple (12) in 1919 reported a decrease in the Addis urea ratio and in the excretion of phenol-sulphonphthalein in dogs with simple ileal obstruction or with closed loop of ileum and concluded that the increase in blood N.P.N. could be due in part to this factor. Brown and co-workers (13) in 1923, reported albuminuria, a decrease in dye excretion and a pathologic picture of toxic nephritis which was particularly associated with gastric tetany, in clinical cases of obstruction. Somewhat recently, Rohland (14) has reported very marked morphological changes in the kidneys of patients who had had pyloric stenosis. There was necrosis and calcification in the renal cortex. It was very uncertain as to the actual responsibility of bowel obstruction, itself, for these anatomic changes.

The most likely basis for the renal insufficiency in our rabbits is the diminished circulatory volume. This was first reported for intestinal obstruction by Herrin and Meek (10) and recently reported to occur in rabbits with bowel obstruction by Ender and Herrin (15). The physical appearance of the rabbits, their susceptibility to blood withdrawal and their low oxygen consumption are additional evidence of circulatory failure in obstruction. Gömöri and Podhradszky (16) measured the plasma colloidal osmotic pressure and arterial pressure in cats with pyloric obstruction and found the effective filtration pressure much reduced. This they pointed out would reduce kidney function. In other experiments, Gömöri and co-workers (17) measured the blood flow through the carotid of cats with pyloric obstruction and found it half the normal. Using renal clearances, Corcoran and Page (18) found renal blood flow in dogs was reduced by hypotension due to bleeding and glomerular filtration was disproportionately decreased. Similar findings of the effects of hemorrhage upon renal blood flow have been reported by other workers (19). Since a reduction of the circulating blood volume by hemorrhage had this effect upon the renal blood flow, one might expect a diminution of blood volume by bowel obstruction to have a similar effect. All of these considerations emphasize inability to maintain adequate renal functions when the circulation is failing. The cause is probably physiological rather than anatomical.

The data also indicate that in about a third of the rabbits of tables 1 and 2 there is an increased rate of urea production. This, although in the past has been attributed to a toxin from the obstructed bowel, might be due to other factors. The loss of extra-cellular fluid and impaired circulation reported (15) to occur in obstructed rabbits might be a factor. Darrow and Yannet (20) produced a deficit of extra-cellular electrolyte in dogs by the intraperitoneal injection of 5 per cent glucose and its removal 4 hours later. This loss differs from that occurring in bowel obstruction in that electrolyte is lost without significant loss of water. Also, in the 7 days of depletion the dogs refused to eat, so that starvation was a factor. However, in 3 of their experiments there was a considerable

negative nitrogen balance and in a fourth there was little nitrogen loss. On the other hand, Kerpel-Fronius (21) used this procedure with rabbits and concluded that in this type of dehydration, which is due to a primary salt loss, there was not increased destruction of body protein because the urinary nitrogen was not increased above that of a fasting period of the same length. However, the data he presented are limited and he made no mention of retention of urea in the body as might well have occurred. Black, McCance and Young (22) studied dehydration in 2 human subjects who drank no water for 3 and 4 days respectively but consumed a dry diet, adequate in protein, salt and calories. The evidence indicated no change in blood volume and the plasma sodium increased. Both subjects incurred a negative water balance of over 3500 cc. so that the volume of extra-cellular fluid must have decreased. This picture therefore presents similarities and differences from that of obstruction. The dehydration increased the average daily excretion of urea by slightly over 3 grams in one and almost 6 grams in the other subject. The writers mention that this might be due to a breakdown of tissue protein. The literature, therefore, indicates that a loss of water or loss of electrolyte without any other apparent complications may result in greater urea production than under normal conditions. Since both are lost in bowel obstruction this might be responsible for the increased urea production in some of these rabbits. It is admitted, however, that this paper has no data opposing the idea that a toxin from the obstructed bowel might be the responsible agent.

#### SUMMARY

Under ether anesthesia intestinal obstruction was produced aseptically in rabbits by a soft ligature 15 cm. below the pylorus. Six of 17 rabbits produced urea at a greater rate during the entire period of obstruction than they excreted urea in a similar period under normal conditions. However, in only 3 animals was the rate more than 17 per cent above the normal. In 11 the decrease ranged from 0.7 to 65 per cent.

The rate of urea excretion during the period of obstruction in the 18 rabbits used was in all cases less than their normal and in all but two very much less than normal. This is in agreement with the anuria or oliguria in obstructed rabbits as reported by Gamble and McIver. In six of the rabbits, the urea excretion, during the first 7 hours of obstruction, remained 90 per cent or more of their normal. In 4 others, after 3 to 5 hours of obstruction, the urea excretion was at least 65 per cent of their normal. This indicated that the renal failure was hardly due to the operative procedure or the obstruction itself but rather was caused by the later consequences of obstruction.

Distention of Thiry fistulae in 3 normal conscious dogs was found to have no significant effect upon their urea and creatinine clearances or rate of urine flow. There was no evidence of increased tubular absorption of urea. These data suggest that the distention of the bowel in the obstructed rabbits would not cause the renal failure.

The skin of the ears of the obstructed rabbits, after 5 or more hours of obstruction, became blanched and cold and showed a reduced hyperemic response to

rubbing. This evidence of cutaneous constriction became aggravated as the period of obstruction increased. Their lips showed cyanosis. The oxygen consumption was greatly reduced by the mid-point of the period of obstruction and it became lower, later in their course. These observations indicate a marked circulatory failure and this might well be the cause of the renal failure to excrete urea. When physiological saline was injected intravenously the urea excretion approximated normal.

These studies emphasize renal failure of urea excretion as the cause for the azotemia of intestinal obstruction. Even in the few animals which produced urea at a greater rate than normal the additional urea produced would be readily excreted by kidneys with adequate circulation.

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# OXYGEN CONSUMPTION OF ERYTHROCYTES OF ADRENALECTOMIZED RATS TREATED WITH VARIOUS LIFE-SUSTAINING SUBSTANCES<sup>1</sup>

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The respiratory metabolism of erythrocytes has never been studied from the standpoint of adrenocortical insufficiency. In fact, the mammalian red cell, being non-nucleated and, indeed, a highly specialized cell, appears to have been purposely avoided on physiological as well as on morphological grounds.

One factor which tends to deter the use of non-nucleated red cells for respiratory studies is the conclusion, persisting from the early literature, that the oxygen consumption is either negligible (25, 15, 8, 5, 13) or non-existent (28). However, it is generally agreed that there is a direct relation between the oxygen consumption of a sample of erythrocytes and its reticulocyte count (24, 15, 8, 6, 28, 16).

There is a parallelism not only between the rate of oxygen uptake and the reticulocyte/erythrocyte ratio but also between the oxygen uptake and the presence of nuclear material within the erythrocyte, since nucleated red cells of birds (25, 26, 27, 18) and reptiles (18) have a relatively high rate of oxygen consumption. It should be pointed out that "the reticulum cannot be demonstrated to contain either desoxyribose or ribose nucleic acid and hence cannot be linked histochemically with the nucleus" (17). Indeed, the work of Watson and Clarke (22) and of others (16) indicates that the reticulum represents an intermediary stage (protoporphyrin) in the synthesis of hemoglobin in these primitive cells.

Contrary to the negative results cited for the oxygen consumption of erythrocytes, Ramsey and Warren (18, 19) conclude, after a careful series of experiments, that non-nucleated, as well as nucleated, red cells "have been found to respire at rates comparable to that of other resting tissue." The work to be reported here supports their conclusions. The contrary results prior to the studies of Ramsey and Warren have been due, at least in part, to the degree of dilution of the red cell suspension and to the technique employed in measuring oxygen uptake.

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**METHODS.** The oxygen uptake of red cells was studied by the Warburg manometric technique. The manometers were oscillated through an amplitude of 3.9 cm. and at a rate of 62 cycles per minute. The respiration flasks were of about 15 cc. capacity. A respiration flask contained 0.3 cc. of Krebs'-phosphate-Ringer's solution (24) in one side-arm and 0.2 cc. of 0.5M NaOH in the central well. The main chamber contained 2.5 cc. of 50 per cent red cell suspension. The manometers were filled with oxygen and equilibrated at the temperature of the bath ( $37.5 \pm 0.01^\circ\text{C}.$ ) for 15 minutes before the taps were closed.

Male white rats, purchased from a supply house in Indiana, were kept under laboratory conditions for at least one week prior to experimental use. They were fed Purina Dog Chow and tap water unless otherwise stated. The rats used in these experiments weighed between 80 and 120 grams. All rats employed for experimental purposes may be divided into 5 experimental groups (see table 1): 1, unoperated (control) rats; 2, adrenalectomized rats; and adrenalectomized rats that were treated with either 3, whole adreno-cortical extract<sup>5</sup> (W.A.C.); 4, desoxycorticosterone<sup>5</sup> (D.O.C.); or 5, 1 per cent NaCl.

Rats were bilaterally adrenalectomized during a single operation while under light ether anesthesia. All operated and extract-treated rats were used, unless otherwise stated, after the fourth day following adrenalectomy. Those operated animals receiving "extract" were treated as follows: Group 5 was given 1 per cent NaCl in drinking water *ad libitum*; otherwise, these rats were treated as were groups 1 and 2. Group 4 was injected with 0.5 cc. D.O.C. (= 1 mgm. in 10 per cent ethyl alcohol) twice/day/rat. Group 3 received 0.2 cc. W.A.C. twice/day/rat. On the evening prior to the morning of sacrifice, each rat received a double-strength dose. One cubic centimeter of this extract contained 150 grams of beef glands. It might be added that, in connection with another problem, a different group of adrenalectomized rats, under the same experimental conditions, was injected with 1 cc. of Upjohn's adrenal cortex extract twice/day/rat without any change being observed in the  $Q_{O_2}$  values for their erythrocytes as compared with the erythrocytes from adrenalectomized rats treated with 0.2 cc. doses of our W.A.C. extract. These data are not included in the results to be presented in this paper.

Blood was taken by cardiac puncture, in the presence of powdered heparin. For each series of experiments (ca. 5 to 6 manometers), blood from 6 to 8 rats, except for some NaCl-fed rats, was pooled. The pooled blood was always centrifuged to constant volume. The centrifuge used in these experiments had a head 6 in. in radius and a rotational velocity of 1500 r./m. when loaded. The plasma was removed after centrifugation; the red cells were washed twice in Krebs' solution; the resulting suspension was centrifuged; and the supernatant solution pipetted. No deliberate attempt was made to remove all of the leucocytes. However, the leucocytes, being of lighter density than the red

<sup>5</sup> We wish to thank Dr. Oliver Kamm of Parke, Davis and Company for the adrenal glands; Dr. R. D. Shaner of Roche-Organon, Inc. for the desoxycorticosterone; and Prof. F. A. Hartman and Dr. J. S. Thatcher for their part in supplying us with the prepared extract.

cells, tend to stratify centripetally to them and this layer of white cells was purposely removed during the pipettings. Finally, the erythrocytes were re-suspended in their own volume of Krebs' solution. Precaution was taken to assure a homogeneous suspension of red cells and, from this suspension, 2.5 cc. samples were placed in respiration flasks and weighing bottles. The latter

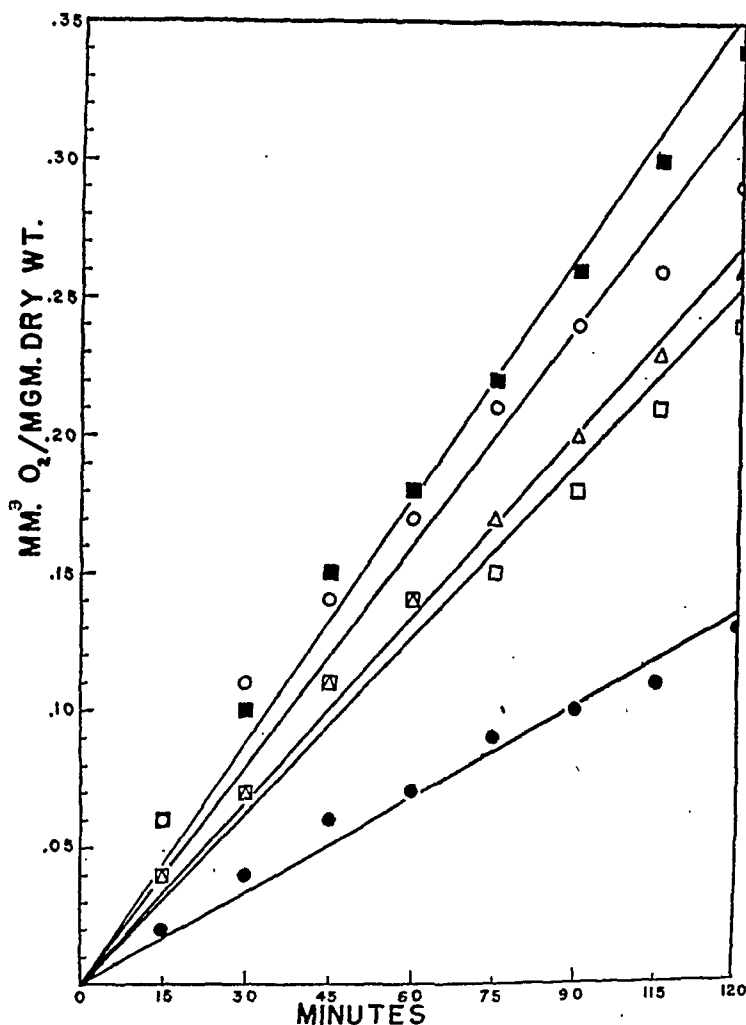


Fig. 1. Comparison of mean oxygen consumption in  $\text{mm}^3/\text{hr./mgm.}$  (dry) weight of washed erythrocytes (ordinates) plotted as functions of time in minutes *in vitro*. Characters represent: ○ = normal rats, ● = adrenalectomized rats, and adrenalectomized rats treated with either ■ = D.O.C., □ = W.A.C., or △ = 1 per cent NaCl in drinking water. See text for methods used to standardize experiment.

samples were placed in a drying oven ( $105\text{--}110^\circ\text{C.}$ ) for 24 hours in order to determine their respective dry weights. From the total dry weight was subtracted the weight of the salts present in an equal volume of Krebs' solution used to bathe the cells. This gave the dry weight of the tissue. The oxygen consumption of the tissue is expressed as  $Q_{O_2}$  (i.e.,  $\text{mm}^3$  of oxygen consumed/hr./mgm. dry weight of tissue).

RESULTS. The results obtained for the oxygen consumption of red cells representative of the five experimental groups of rats are to be found in figure 1. Here is plotted the mm.<sup>3</sup> of oxygen consumed/mgm. (dry weight) of red cells (ordinates) as a function of time in 15-minute intervals during the initial 2-hour period *in vitro* (abscissae).

An inspection of the various curves shows that the respective rates of oxygen consumption for the five experimental groups of red cells have been averaged over a 2-hour period. On closer scrutiny, it is found that the oxygen consumption for a given curve is of higher value during the first hour than during the

TABLE 1

Summary of the mean values for the oxygen consumption ( $Q_{O_2}$ ) for erythrocytes of normal, of adrenalectomized, and of adrenalectomized rats treated post-operatively with either whole adrenocortical extract (W.A.C.), desoxyz corticosterone (D.O.C.), or 1% NaCl

RATS (MALE, 50-120 GM., POST- ABSORPTIVE STATE)	UNOPERATED	ADRENALECTO- MIZED	ADRENALECTOMIZED—TREATED WITH		
			W.A.C.	D.O.C.	NaCl
Experimental groups....	1	2	3	4	5
No. of rats used.....	15	46	37	24	32
No. of experiments.....	18	18	18	37	26
Mean $Q_{O_2}$ values					
*1 hour.....	0.15	0.04	0.14	0.19	0.15
2 hour.....	0.13	0.03	0.09	0.18	0.11
% difference.....	-13	-25	-36	-5	-27
*S.D. $\pm$ .....	0.061	0.022	0.052	0.099	0.074
*S.E. $\pm$ .....	0.014	0.005	0.001	0.016	0.014
*"t" values.....		7.4	0.54	0.28	0.00
		H.S.	N.S.	N.S.	N.S.

$Q_{O_2}$  = -mm.<sup>3</sup> O<sub>2</sub> consumed/hr/mgm. (dry) weight of tissue. Cells washed twice, centrifuged, and made to 50% cell suspension by volume with Krebs'-phosphate-Ringer's solution (pH 7.4). Samples = 2.5 cc. Temperature 37.5°C.

S.D. = Standard deviation. S.E. = Standard error of a given mean  $Q_{O_2}$  value.

"t" = ratio of statistical comparison of the difference between two mean  $Q_{O_2}$  values/  
 $\sqrt{\Sigma}$  respective standard deviations.

Statistically: H.S. = highly significant; N.S. = not significant.

\* = Related statistics.

second hour *in vitro*. This fact becomes more lucid when the  $Q_{O_2}$  for the first and second hours are compared in table 1.

The more statistically pertinent data are recorded in table 1. The first horizontal column designates the treatment accorded the five groups of rats employed in these experiments; while the first vertical column shows, in descending order, the general characteristics of the experimental material, the numerical identification of each group, the total numbers of rats used to determine a given mean value for the  $Q_{O_2}$ , the number of individual experiments performed, the mean  $Q_{O_2}$  values for the first and second hours respectively during respirometry, the per cent difference between these latter two values,

and the standard deviation (S.D.) and the standard error (S.E.) respectively of any given mean  $Q_{O_2}$ , as based on data for the first hour and, finally, a statistical comparison ("t" values) of the ratio of the differences between any given mean and the mean for the unoperated (control) group/square of the sum of their respective standard deviations.

The results show that there is no statistical significance (see "t" values in table 1) between the mean  $Q_{O_2}$  values for red cells obtained respectively from adrenalectomized rats treated post-operatively with any one of the various life-sustaining substances (groups 3, 4 and 5) and from unoperated rats (group 1). In other words, the red cells of adrenalectomized rats treated post-operatively with either W.A.C., D.O.C., or NaCl give the same  $Q_{O_2}$  as do red cells obtained from unoperated (control) rats. There is a very significant decrease ( $-73$  per cent) in the mean  $Q_{O_2}$  for red cells obtained from adrenalectomized rats (group 2) when compared with the mean  $Q_{O_2}$  for the red cells of unoperated rats (group 1). This same decrease is obtainable at the end of the second and subsequent days, but not at the end of the first day following adrenalectomy. Incidentally, one may note an apparent difference between the mean  $Q_{O_2}$  values for the red cells of adrenalectomized rats treated post-operatively with D.O.C. (group 4) and with W.A.C. (group 3). There is, however, no significance between these values when a statistical comparison is made.

It is interesting to point out that a group of adrenalectomized rats treated post-operatively with 1 cc. of Upjohn adrenal cortical extract (see Methods) twice/day/rat gave mean  $Q_{O_2}$  for red cells that were statistically no different from the  $Q_{O_2}$  for the red cells of adrenalectomized rats treated post-operatively with either 0.2 cc. of our W.A.C. or 0.5 mgm. D.O.C. under the same experimental conditions. From a statistical standpoint, it appears that D.O.C. and W.A.C. restore the respiratory metabolism of red cells of adrenalectomized rats to "normal" but to no higher  $Q_{O_2}$  value. Thus the respiration of the red cells of these rats appears to be independent of the dosage-strength of D.O.C. and W.A.C. once the maintenance level has been reached.

Reticulocyte counts were made by the wet method after the cells were stained with brilliant cresyl blue. Two cubic centimeter samples of whole blood per rat were obtained under experimental conditions as nearly as possible like those employed in preparing the red cells for respirometry. The data obtained from these studies are presented in the following order: the experimental group of rats contributing blood samples, the reticulocyte/matured erythrocytes ratio (count), the per cent reticulocytes present in the total counts, and, in parentheses, the number of rats donating to the blood pool: normal rats 39/4274—0.96 per cent (10); adrenalectomized rats 29/2858—1.01 per cent (11); adrenalectomized rats treated post-operatively with either W.A.C. 44/5522—0.71 per cent (5), or D.O.C. 60/6766—0.88 per cent (6). When the various mean values for the reticulocyte/matured erythrocyte counts are treated statistically by the method of chi-square, it is concluded that there is no significance between the mean values for any one of the experimental groups (2, 3, 4) and the mean value of the control (group 1). These studies were made on whole blood since, to our knowledge, there is no report as to the effect of total ablation of the

adrenals on the reticulocyte count. Of course, in light of the well-known effect of reticulocytes on the rate of oxygen uptake, it is pertinent to know how our experimental technique involving washings and centrifugations affects the reticulocyte/erythrocyte ratio. In two studies it was found that there is a reduction in reticulocytes by slightly more than 100 per cent. This suggests that our reticulocyte counts on rats are well within the range reported by Ramsey and Warren (19) for rabbits. This observation supports that of Key (16) who found that reticulocytes move centripetally in a gravitational field and this suggests a lower specific gravity for these cells than for the erythrocytes.

It was noted that there is a change in hematocrit volume of a sample of red cells when a comparison is made before and after washing in Krebs' solution and on subsequent centrifuging to constant volume. Samples of red cells taken from the five experimental groups of rats gave the following changes in volume when placed in isosmotic Krebs' solutions: Group 1, 0.0 per cent (standard); 2, -1.85 per cent; 3, +3.28 per cent; 4, +0.30 per cent; and 5, +1.25 per cent.

DISCUSSION. The effect of adrenalectomy and the action of various cortin-like substances on the oxygen consumption of red cells of adrenalectomized rats has been studied for the first time. Furthermore, these results on rats support the results of Ramsey and Warren on rabbits and man (18, 19), that there is a measurable oxygen consumption for non-nucleated red cells. Undoubtedly, non-nucleated red cells of other species will be found to give recordable respiratory rates.

Since the studies (3, 12) showing a decrease in basal metabolic rate following adrenalectomy, there has been reported either a decrease or no change in  $Q_{O_2}$  for the isolated tissues of adrenalectomized animals when a comparison is made with their controls. The decrease in tissue  $Q_{O_2}$  reported here for the red cells of adrenalectomized rats is in line with those of other workers who find a similar decrease. The results for various other tissues of the adrenalectomized animals recorded here are for rats unless otherwise stated: brain—64 per cent based on wet weight (11), but these results were not confirmed (4, 21); kidney—21 per cent (21), and -18 per cent when "acutely insufficient" (20); descending aorta—29 per cent (1). In contrast to these results, the following tissues from adrenalectomized animals gave no change in  $Q_{O_2}$ : testicles, liver and kidney, based on wet weight (11); brain (4, 21); skeletal muscle (4); diaphragm (21); sciatic nerves (1).

At the close of the first hour of respirometry, the decrease of 73 per cent in mean  $Q_{O_2}$  for erythrocytes of adrenalectomized rats as compared with their controls (group 1) appears to be the lowest value yet recorded in this field of tissue respiration. However, it is noted that the comparison between the  $Q_{O_2}$  for the erythrocytes and the  $Q_{O_2}$  values for the aforementioned tissues differs as regards time. Most studies on isolated tissues of adrenalectomized animals have begun with the close of the first hour following their removal; but, with erythrocytes, time is consumed in washings and in centrifugations, so our zero reading begins with the close of the second hour following heart puncture. Therefore, our  $Q_{O_2}$  values must be considered proportionally lower than they would be at the close of the earlier hour.

The relatively great decrease in the  $Q_{O_2}$  for red cells *in vitro* at the end of the second hour has already been noted (18). However, the far greater decrease (ca. 25 per cent) in  $Q_{O_2}$  for the erythrocytes of adrenalectomized as compared with unoperated rats has not been noted in the case of other tissues. Apparently, treating the adrenalectomized rats with 1 per cent NaCl in drinking water or with maintenance doses of W.A.C. does not materially alter the results. The erythrocytes from D.O.C.-treated adrenalectomized rats show the least decrement in  $Q_{O_2}$ . It will be noted that the dosage-strength of this substance was considerably more than necessary for maintenance. It would be interesting to investigate comparable dosage-strengths of W.A.C. under similar conditions.

It was noted that red cells from the 5 experimental groups, when placed in isosmotic Krebs' solution, showed a decreased hematocrit volume in the case of group 2 and an increased hematocrit volume in the case of groups 3 and 5, as compared with groups 1 and 4. While determining the cell volume on the basis of centrifuging to constant volume is subject to considerable error, the results suggest that the red cells from adrenalectomized rats tend to lose, while those from adrenalectomized rats treated with either NaCl or W.A.C. tend to gain in volume when immersed in Krebs' solution isosmotic for normal red cells. It has been known that, upon adrenalectomy, water tends to shift into skeletal muscle (9, 4, 2) and into erythrocytes (9, 10). Thus, the water-loaded red cell appears to give up its water to the bathing medium. This same condition has been found in the case of the skeletal muscles of adrenalectomized frogs (2). The explanation for this activity still begs solution.

It is noted that adrenalectomy has no effect on the reticulocyte count. These results support the observation on Addisonians who were found to show no typical difference in reticulocyte count as compared with normal patients (14). Ramsey and Warren (19) point out that, assuming a 0.5 per cent reticulocyte concentration in a blood sample, 90 per cent of the oxygen consumption is ascribed to orthochromatic erythrocytes. The reticulocyte count in our experiments is well within this figure, so, assuming the results on the rabbit to hold for the rat, one may conclude that the oxygen uptake is due almost entirely to erythrocytes.

#### SUMMARY

1. There is a definite and measurable respiratory metabolism of the non-nucleated red cells of the rat. This work confirms the results of Ramsey and Warren (18, 19).

2. The removal of both adrenals in the rat produces a decrease of 73 per cent in the mean values of  $O_2$  uptake (expressed as  $Q_{O_2}$ ). A decrease in  $Q_{O_2}$  is not identifiable at the end of the first day but is obtained at the end of the second day, and, thereafter, apparently remains at this level until the terminal stage.

3. Either whole adreno-cortical extract (W.A.C.), desoxycorticosterone (D.O.C.), or 1 per cent NaCl added to drinking water will return the  $Q_{O_2}$  of the erythrocytes of adrenalectomized rats to the same statistical value characteristic of the controls.

4. Doses of extract (W.A.C. and D.O.C.) stronger than necessary for maintenance of the adrenalectomized rat do not raise the  $Q_{O_2}$  for the red cells above that value characteristic for the controls (unoperated rats). On the other hand, D.O.C., at least, tends to prevent the relatively great decrease in  $Q_{O_2}$  for red cells of adrenalectomized rats with time *in vitro*.

5. Reticulocyte counts were made on adrenalectomized, and on adrenalectomized rats treated with W.A.C. and D.O.C. Neither of these conditions modified the reticulocyte count. This work confirms the observation (14) that Addisonians suffer no change in their reticulocyte count.

6. Following adrenalectomy, there is an apparent increase in the water load of the red cell since isosmotic Krebs' solution behaves as though it were hypertonic to the red cell; while the red cells from adrenalectomized rats treated with either NaCl or D.O.C. behaved as though the Krebs' solution were hypotonic. The former result for the behavior of red cells after adrenalectomy when immersed in Krebs' solution is in line with similar results obtained in skeletal muscles of adrenalectomized frogs when immersed in normal Ringer's solution (2).

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# THE RELATIONSHIP OF THE ADRENAL GLANDS TO CHANGES IN URINARY NON-PROTEIN NITROGEN FOLLOWING MULTIPLE FRACTURES IN THE FORCE-FED RAT

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This is one of a series of studies on the relationship of adrenal function to some of the metabolic adjustments which occur during stress. The administration of large amounts of adrenal extracts or certain steroids or the stimulation of the adrenal cortices by adrenocorticotrophic hormone in the intact rat causes a significant increase in the non-protein nitrogen of the urine. The secretory activity of the adrenal cortices is thought to be increased in every type of stress. Laboratory animals as well as patients show an increase in the urinary excretion of non-protein nitrogen following fractures and other types of stress. This, in brief, is the basis for the hypothesis that the increased loss of nitrogen which follows fractures is caused by an increase in the secretory activity of the adrenal cortices. In the present study it was shown that the presence of the adrenal cortical hormone is essential for the response to occur but that a change in the secretory activity of the adrenals or in amount of adrenal hormones is not essential for increased nitrogen loss following fractures.

**METHODS.** Male rats of the Sprague-Dawley strain which were completely free from infections were used. The stock diet was Purina Dog Chow. When the rats reached a weight of approximately 300 grams they were adapted to the forced-feeding of a medium carbohydrate diet (table 1) administered by stomach tube each morning (8:30 to 9:15 a.m.) and afternoon (4:15 to 5:00 p.m.). The technique of forced-feeding and the diet used were modifications of those described by Reinecke, Ball and Samuels (1). The rats were brought to a full feeding on the fifth day. After the rats had been on a full feeding for two weeks they were adrenalectomized by the procedure described by Ingle and Griffith (2). In the control animals the adrenal glands were exposed but not damaged. Asepsis was successfully maintained in these operations. The adrenal cortical extract was prepared from beef glands and represented 40 grams per cc. It contained 0.9 per cent sodium chloride and was free from alcohol. It was injected in divided doses each morning and afternoon in amounts of 4 cc. per rat per day. Fractures were made under ether anesthesia.

The animals were housed in an air-conditioned room in which the temperature was maintained at 74 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation. Twenty-four-hour samples of urine were collected at the same hour each day and preserved with thymol and citric acid (1 gram per sample) to insure the acidity of the urine for nitrogen analysis. The determination of urinary non-protein nitrogen was by the micro-Kjeldahl procedure as follows: proteins were precipitated as the salts of tungstic acid by the Folin-Wu pro-

cedure. The organic matter was oxidized by sulfuric acid and hydrogen peroxide. The ammonia was distilled off into a standard acid solution and titrated with standard base.

**EXPERIMENTS AND RESULTS.** In experiment 1 (fig. 1), 12 rats were maintained on a medium carbohydrate diet for two weeks at which time six rats were adrenalectomized and six were sham-operated. All of the animals received a 0.9 per cent solution of sodium chloride to drink during all phases of the experiment. Two weeks following operation all of the animals were subjected to

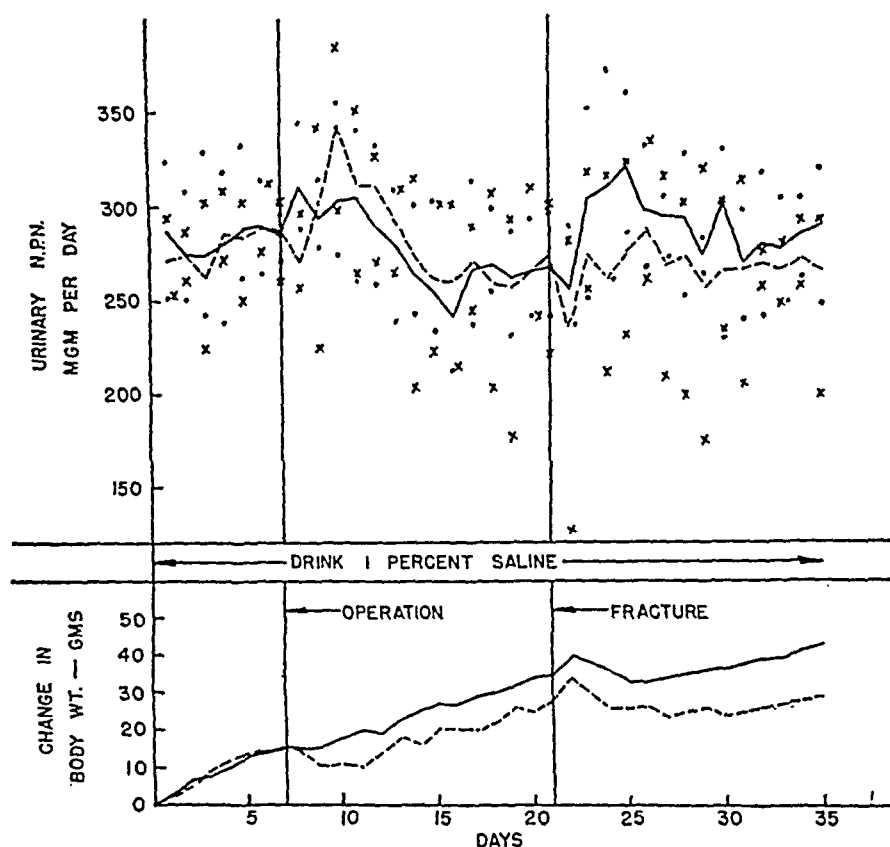


Fig. 1. The effect of operation and of fractures on the urinary non-protein nitrogen of six pairs of sham-operated (· —) and saline-treated adrenalectomized (x - - -) rats. Averages and range of values.

fracture of the knee-joint and tibia of the right hind leg. No hormone was used in this experiment.

During the first post-operative day the adrenalectomized rats excreted less nitrogen than their controls; but by the third day there was a rise in urinary nitrogen which was sustained for several days and was greater in the adrenalectomized rats than in the non-adrenalectomized group. During the two weeks of post-operative study the level of urinary nitrogen of the adrenalectomized series did not decrease significantly below their own preoperative level or the level of the control series. These results parallel those reported by Ingle

and Oberle (3) from a similar experiment. Following the fractures there was a fall in the level of urinary nitrogen for 24 hours in the adrenalectomized rats and then recovery, but there was little or no elevation in the level of excretion over the pre-stress level. In contrast, there was a striking increase in the excretion of nitrogen by the non-adrenalectomized rats which was sustained for several days. All of the rats showed a gain in weight during the first 24 hours which was probably due to retention of fluid in the area of the fracture. The

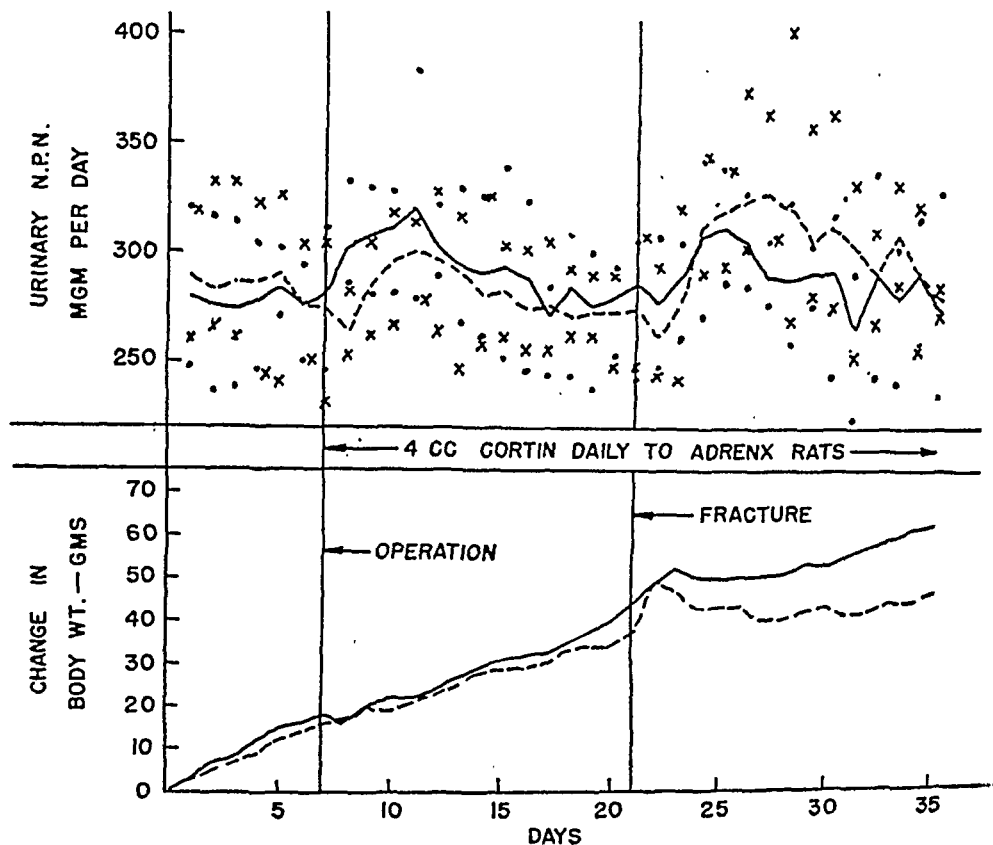


Fig. 2. The effect of operation and of fractures on the urinary non-protein nitrogen of six pairs of sham-operated ( $\cdot$  —) and cortin-treated adrenalectomized ( $x$  - - -) rats. Averages and range of values.

edema rapidly disappeared and all of the rats lost weight. The depression of weight was greater in the adrenalectomized animals than in their controls.

Experiment 2 (fig. 2) was identical with experiment 1 except that treatment with saline was omitted and the adrenalectomized rats were treated with 4 cc. daily of beef adrenal extract from the time of operation to the end of the experiment four weeks later. In this experiment the rise in nitrogen excretion following operation was less in the adrenalectomized cortin-treated animals than in their controls and less than was shown by the saline-treated adrenalectomized rats of experiment 1. Following the fracturing of the tibia and knee-joint the adrenalectomized cortin-treated rats excreted less nitrogen during the

first 24 hours but thereafter showed a rise which was even more marked than the rise which occurred in the non-adrenalectomized series following fractures. The edema of the injured limb was much greater in the cortin-treated adrenalectomized animals than in the non-adrenalectomized animals. This was reflected in the temporary gain in weight by the adrenalectomized group (fig. 2).

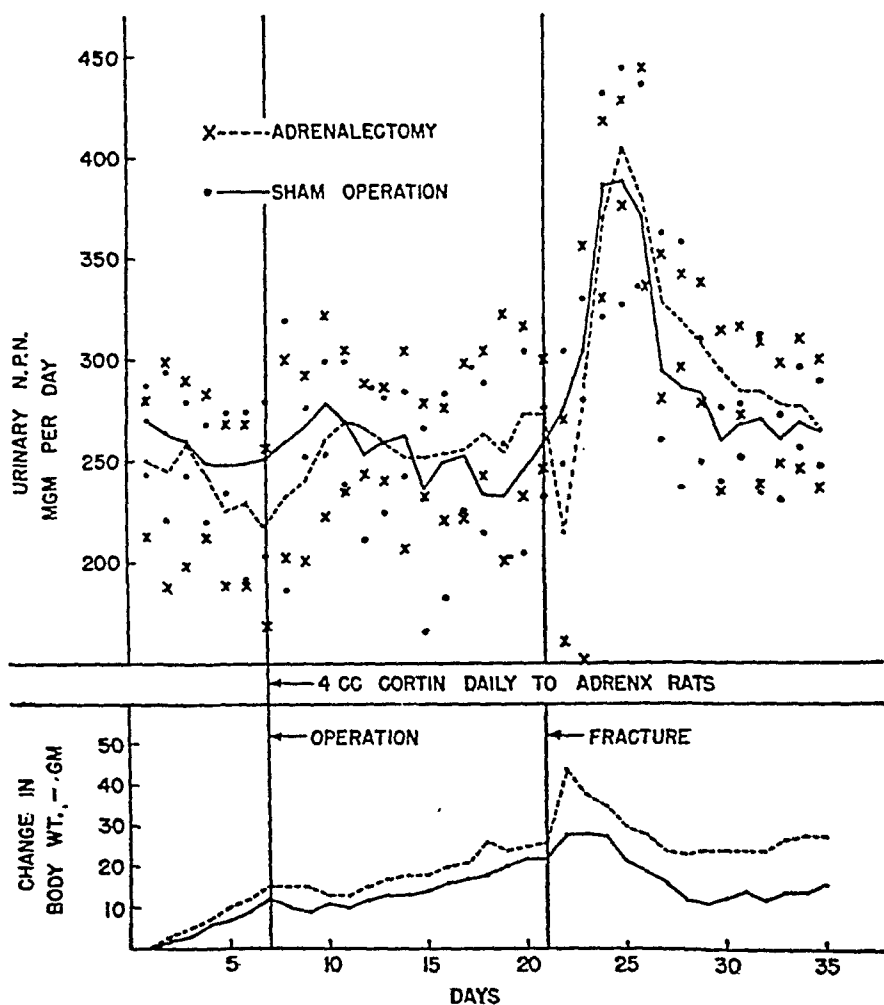


Fig. 3. The effect of operation and of fractures on the urinary non-protein nitrogen of six pairs of sham-operated (· —) and cortin-treated adrenalectomized (x - - -) rats. Averages and range of values.

Subsequently, the gains in weight by the adrenalectomized animals were less than in the control series.

Experiment 3 (fig. 3) was identical with experiment 2 except that the tibia, femur and knee-joint of both hind legs were fractured in each rat. The results paralleled those of experiment 2 except that the post-stress rise in nitrogen excretion was greater in each group and the retention of fluid in the injured limbs was more marked in the adrenalectomized cortin-treated animals. During

the first 36 hours following the fractures the cortin-treated rats had the appearance of being "sick" and in mild "shock." Improvement was rapid.

**DISCUSSION.** In the cortin-treated rats (figs. 2 and 3) the rise in urinary nitrogen following adrenalectomy was not as great as that observed in the saline-treated adrenalectomized animals in this (fig. 1) and earlier studies (3). Does the presence of the adrenal glands or treatment with cortin tend to suppress the rise in urinary nitrogen below the response of the adrenalectomized rat treated with saline only? Additional study will be required.

The results of this study show that whereas the effect of a fracture in causing negative nitrogen balance is not manifest in the absence of the adrenal cortical hormones (fig. 1) it does occur in the presence of a steady intake of cortin when the adrenal cortices are absent (figs. 2 and 3). These findings are like those which we obtained (4) in testing the hypothesis that the diabetogenic activity of diethylstilbestrol might be caused by stimulation of secretory activity of the adrenal cortices. There also, the presence of cortin was required for a manifestation of the diabetogenic activity of diethylstilbestrol but the response occurred in the presence of a steady intake of cortin. All of these results re-emphasize that a discrimination must be made between the essentiality of a hormone for a response to occur and its possible rôle in specifically causing the response by secreting an increased amount of the hormone.

In all of our experiments thus far there has been a difference in the nitrogen excretion of non-adrenalectomized rats and either cortin- or saline-treated adrenalectomized rats during the first 24 hours following an operation or a fracture. May this reflect a difference in the dissolution of lymphoid and lymphocyte tissue (5) which does seem to occur rapidly and extensively only in response to increased amounts of adrenal cortical hormones? The difference may also be non-specific.

It has been proven beyond any reasonable doubt that the secretory activity of the adrenal cortices is increased following stress. What purpose does this serve? It plays some important rôle in maintaining resistance to stress, but by what means? Some of the biologic changes following stress resemble changes which can be induced by overdosage with the adrenal hormones but, as in the present experiment, the metabolic adjustments to damage may not be directly caused by the increase in adrenal activity. One of us (4) has previously suggested that the increased amount of cortical hormones secreted during stress does not cause "over-dosage" changes in the presence of an increased physiologic need. Rather, the increased secretory activity of the cortex maintains the normality of tissue functions, sustains the reactivity of defense mechanisms and serves to maintain homeostasis rather than to disturb it.

#### SUMMARY

The urinary non-protein nitrogen excretion of adrenalectomized and sham-operated force-fed male rats was studied before and following operation and fractures of the hind legs. In experiment 1, saline was the only therapy used. Following operation the urinary nitrogen increased in each group, higher in the

adrenalectomized; no significant rise followed the breaking of the tibia and knee-joint of one leg in the adrenalectomized rats but a significant rise occurred in the controls. In experiment 2, the adrenalectomized rats were treated with 4 cc. of adrenal cortical extract daily instead of saline. In the adrenalectomized cortin-treated rats the rise in nitrogen excretion following operation was less than in saline-treated adrenalectomized rats. Following fractures there was a marked rise in the nitrogen loss by the rats of each series. Experiment 3 was identical except that the tibia, femur and knee-joint were fractured in each hind leg. The post-stress increase in nitrogen loss was much greater in each series than in the other experiments. In all experiments the loss of nitrogen by adrenalectomized rats was less than for the controls during the first 24 hours following either an operation or fracture. The subsequent response was just as marked in the cortin-treated adrenalectomized rats as in the sham-operated rats.

✓ It is concluded that the negative nitrogen balance which characteristically develops following fractures may require the presence of the adrenal cortical hormones but is not caused specifically by the increase in secretion of the cortical hormones which occurs during stress.

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## THE ISOLATION OF THE CEREBRAL CIRCULATION AND THE PERFUSION OF THE BRAIN IN THE LIVING CAT

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Hitherto the main obstacle to the development of a brain perfusion method in the living animal has been the failure to isolate completely the cerebral circulation. Earlier brain perfusion experiments were either performed on the severed head (Bouckaert and Jourdan, 1936; Chute and Smyth, 1939) or in others quantitative collection of the blood was impossible owing to the incomplete blockage of the venous outlets of the brain (Schmidt, 1928). It became evident at the beginning of our experiments in 1940 that the main problem in isolating the venous outflow was to gain access to and to occlude the sinusii columnae vertebralis which may carry the entire venous return from the brain, as mentioned already in 1896 by L. Hill. (See also Harris, 1941.)

This paper presents a brain perfusion method based on the complete isolation of the cerebral circulation in the living cat. Brain metabolism experiments are described in which the animals were kept alive and without visible impairment of cerebral functions for over two hours while perfusing their brain with defibrinated heparinised ox blood.

*Anatomical considerations and surgical technique.* Our surgical procedure consists mainly in the isolation and occlusion of the venous outlets from the brain and is supplemented by the ligation of certain arteries. In the cat, unlike the monkey (Dumke and Schmidt, 1943; Batson, 1944) the separation of the cerebral from the extracranial circulation on the arterial side is practically impossible, as most, if not all the blood from the carotids reaches the brain via anastomoses between the rete mirabile (Ask Upmark, 1935) of the arteria maxillaris interna and the circle of Willis. The internal carotid is an insignificant vessel, or, according to Davis and Storey (1943) has no lumen at all. The anatomy of the venous outlets from the cat's brain is described here in some detail, not as an original contribution but rather as a reminder in view of some of the past errors which have arisen due to its neglect. Since the completion of our anatomical observations, Batson (1944) has described many of these anatomical details.

Each of the two *sinusii columnae vertebralis* is formed in the atlas by the confluence of two veins issuing from the cranium through the foramen magnum, one dorsally, the other ventrally. Thus, including the vertebral veins, at the level between the occiput and the atlas, there are three pairs of veins with free communication among themselves (within the cranium and the atlas) to ensure effective drainage of the cerebral sinuses. The "spinal venous sinuses" (*sinusii columnae vertebralis*) run the length of the vertebral column communicating

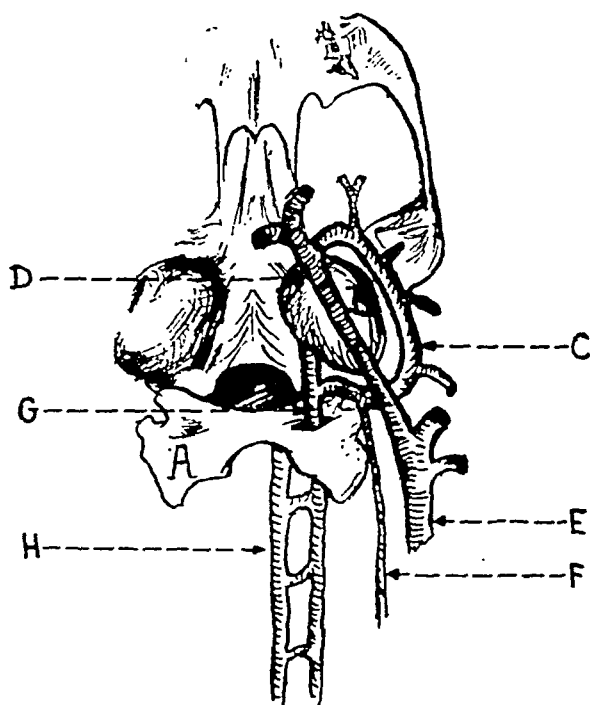


Fig. 1

Fig. 1. The branch connecting the vertebral vein with the external jugular vein and the communications between the vertebral vein and the *sinus columnae vertebralis*. A—atlas; B—bullae tympani; C—communicating branch between the vertebral and posterior facial veins; D—posterior facial vein; E—external jugular vein; F—internal jugular vein; G—vertebral vein; H—*sinus columnae vertebralis*.

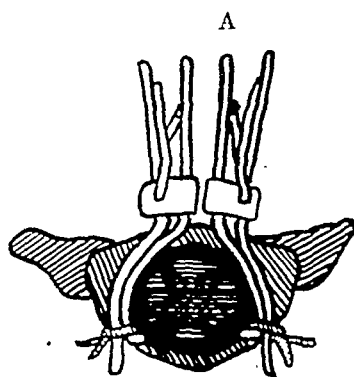


Fig. 2

Fig. 2. The position of the clips compressing the *sinusii columnae vertebralis* between atlas and occiput shown in cross-section. A—ventral side.

at each interspace with the vertebral veins (as shown schematically in fig. 1). After the exit of the latter from the transverse foramen they communicate in their further course with the Azygos system by way of the intercostal veins. Thus, unless the sinuses and the vertebral veins are occluded at one and the same level, blood will escape from one pair of veins to the other by means of their communications at other segments. The spinal venous sinuses also communicate with each other by means of small dorsal connecting branches.

After leaving the skull and before entering the transverse foramen in the atlas the vertebral veins give off a large branch connecting them with the external

jugular veins (fig. 1). This branch after giving off the internal jugular<sup>1</sup> and communicating with its homologue on the opposite side, circles the bulla tympani (receiving in its course 3-4 other small branches) and finally has its outlet into the posterior and/or anterior facial branch of the external jugular vein. We are not aware that this branch has been described elsewhere. Thus, if the external jugulars are ligated, all the extracranial venous blood may enter the vertebral veins by means of this branch, which may carry blood in either direction. In addition to the important venous exits just mentioned there are other small venous communications through the trigeminal fissures between the brain and extracranial tissues. These are excluded from the circulation by occluding the arteries which feed the tissues, from which they originate.

The following branches of the carotid arteries are ligated: a. The superior thyroid and dorsal muscular branches of the common carotids. b. The lingual, external maxillary and submaxillary branches of the external carotids. c. The terminal section of the internal maxillary artery rostral to the anastomotic branches supplying the circle of Willis. This last ligation is performed through the mouth, the artery easily being exposed in the angle formed at the posterior junction of the palatine and maxillary bones and tied caudal to the bifurcation of the aa. infraorbitalis and sphenopalatina.

The vertebral arteries are ligated before their entrance into the transverse foramen of the 6th vertebra. Though there are numerous small muscular branches arising from the vertebral arteries as they course upwards, we have never lost perfusion blood through these branches, possibly because the external jugulars, which collect a large proportion of the muscular venous return from this region, are occluded. A small branch which forms an anastomosis between the vertebral and carotid arteries is ligated together with the vertebral vein on each side. From our experience with a very large number of animals we are satisfied that the anterior spinal artery in the cat is so small that it may always be safely disregarded.

On the basis of numerous injections and perfusion experiments, we may safely conclude that the method described here isolates effectively the cerebral venous outflow so that all the venous blood from the brain may be collected, as described subsequently, with the possible admixture of but insignificant amounts from extracranial tissues.

*The technique of the operation* is as follows: The cat (preferably lean, short-haired and weighing about 3 kgm.) is first anesthetized with ether and then given 70 mgm. of Dial/kgm. intravenously. After the insertion of a tracheal cannula the vertebral arteries and the above mentioned branches of the carotids are ligated, the trachea and esophagus are cut and retroflexed and the anterior occipito-atlantoid ligament and vertebral veins at this level exposed. The branches connecting the vertebral and external jugular veins are tied. The

<sup>1</sup> In cats the internal jugular veins are usually very small. In about  $\frac{1}{3}$  of our cats, one larger internal jugular was found, usually on the left side, and in these cases the vertebral vein on the same side was invariably smaller than usual.

animal is then turned over and the dorsal occipito-atlantoid ligament exposed. The capsule between occiput and atlas is cut as far as possible on both sides. The occipital bone is scraped clean for subsequent insertion of cannulae. The cat is turned over on its back again and a longitudinal incision is made through the middle of the anterior occipito-atlantoid ligament and underlying dura. Both the ventral and dorsal pairs of veins which go to form the spinal sinuses are compressed by specially constructed spring clips as shown in figure 2.

The inner jaw of the open clip is slid between dura and spinal cord through the ventral incision. The outer jaw lies between the atlas and the occiput external to the lateral part of the occipito-atlantoid ligament with its tip emerging dorsally; after closing the clip the cat is again turned over, the dura incised dorsally thus exposing the small hook at the end of its inner jaw to which the protruding end of the outer jaw is firmly tied, with strong silk thread. After administration of heparin to the animal (1000 units/kgm.) a hole is bored through the occipital bone on each side, just above and a little to the side of the occipital condyle thus tapping the transverse sinuses of the occipital bone which in the cat collect the blood from all the cerebral sinuses. Some practice is necessary for the proper placing of these holes and care must be taken not to pierce the meningeal layer of the dura. Metal cannulae are screwed into the holes. (After numerous attempts at using the torcular Herophili for collection of the venous blood this was given up as it is not sufficiently developed in the cat.) After ascertaining that blood is flowing freely through the two cannulae just inserted, the cat is turned on its back and the cannulae are connected to an external jugular vein thus ensuring adequate venous outflow from the brain until the start of perfusion. T-cannulae are inserted into both carotids and the remaining jugular vein, and the vertebral veins are ligated between occiput and atlas. After connecting the arterial and venous cannulae to the perfusion apparatus, perfusion is started and the systemic connections of the carotids and of the jugulars clamped. By changing the position of the clamps it is easy to re-establish the animal's own circulation to the brain. The systemic blood pressure of the animal is recorded with a membrane manometer from a femoral artery and respiration by means of a pneumograph. After finishing the experiment the brain is removed down to the calamus scriptorius and weighed. At this level the sinusii columnae vertebralis were occluded.

*The apparatus.* The brain perfusion apparatus is shown schematically in figure 3. The following parts require special description.

*The pressure regulator* (fig. 4 and *H* in fig. 3) consists of two chambers: *A* and *B*, separated by a membrane about 2 mm. thick made of two sheets of rubber glued together, with a thin steel plate inserted between them at the centre. The upper chamber *A* is connected to the central stump of the carotid artery or to a manometer and syringe through which pressure can be exerted. The lower chamber *B* contains the inlet and outlet tubes (*c*) of the perfusion circuit and an overflow (*d*), the mouth of which is ground to the level of the rim of the lower chamber *B*.

Blood from the pump entering the lower chamber *B* at a pressure exceeding that in *A*, lifts the membrane and opens the overflow, equalising the pressure

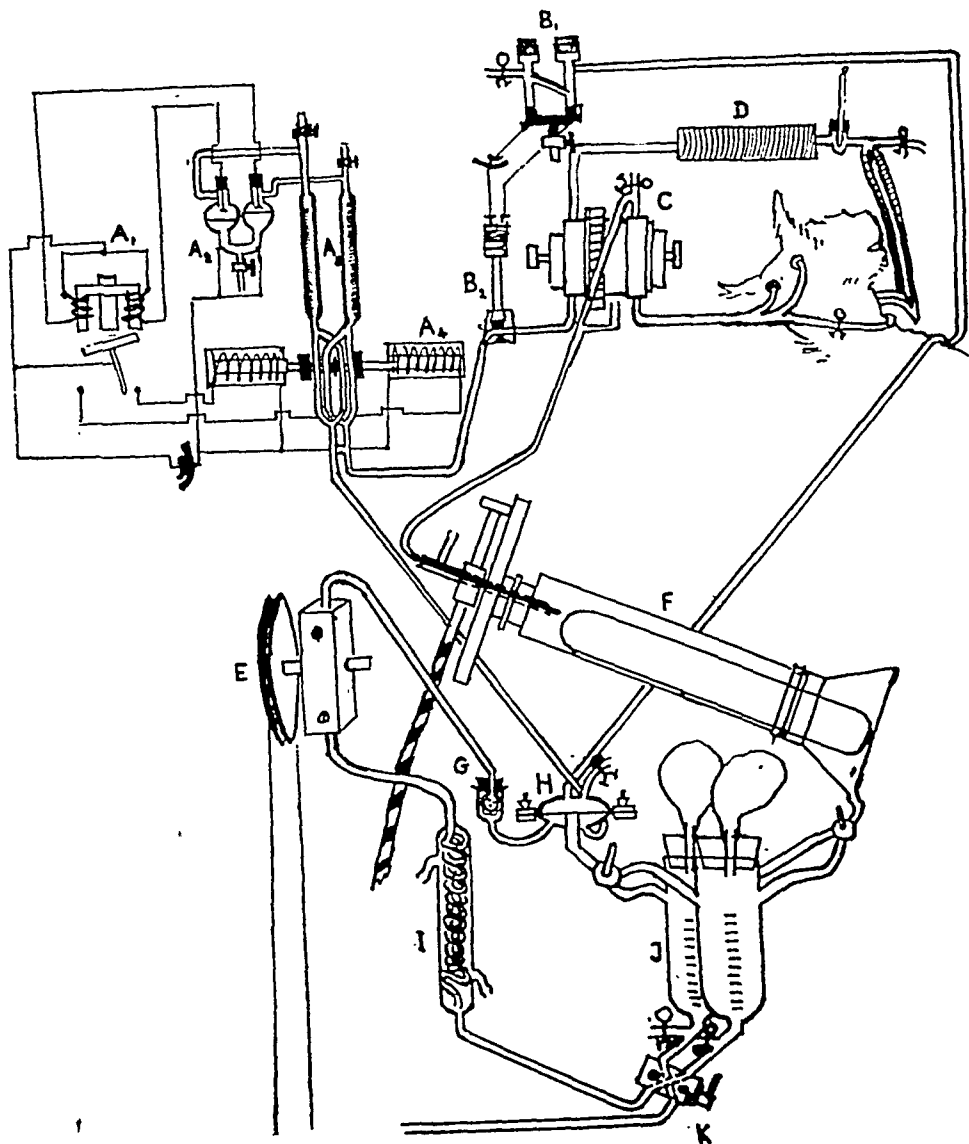


Fig. 3. Diagram of brain perfusion apparatus. *A*<sub>1</sub>—Flowmeter, polarized relay. *A*<sub>2</sub>—Flowmeter, electrolytic interrupter. *A*<sub>3</sub>—Flowmeter, graduated blood pipettes. *A*<sub>4</sub>—Flowmeter, electromagnetic valve. *B*<sub>1</sub>—Pulse frequency regulator, interrupter. *B*<sub>2</sub>—Pulse frequency regulator, electromagnetic valve. *C*—Photoelectric apparatus for measuring A-V oxygen differences. *D*—Heater. *E*—Roller pump. *F*—Oxygenator. *G*—Blood filter. *H*—Pressure regulator. *J*—Graduated blood reservoirs. *K*—Stopcock.

in both chambers. As the upper chamber is connected to the systemic end of the carotid artery and as the systemic blood pressure is still under central control, vasomotor impulses from the brain are able to regulate the perfusion pressure.

The inside diameter of the pressure regulator is 40 mm. and that of the overflow tube 5 mm. The diameter of the overflow tube should be small in proportion to that of the membrane in order to minimise the error due to the fact that the membrane area covering the overflow tube is under pressure only from chamber *A*. Thus the pressure in chamber *B* will exceed that in chamber *A* in proportion to the fraction: surface area of overflow tube/surface area of membrane. In our apparatus with a membrane area of 12.5 cm<sup>2</sup>, and an overflow area of 0.2 cm<sup>2</sup> the pressure difference between chamber *A* and *B* is less than 2 per cent.

The *flowmeter* (*A* in fig. 3) is essentially the instrument described by Montgomery et al. (1934), with electrolyte control of electro-magnetic valves operated by a 10 volt current through a polarised relay. In our apparatus two graduated pipettes are used as blood chambers and the blood is separated from the electrolyte (saturated KCl solution) by air.

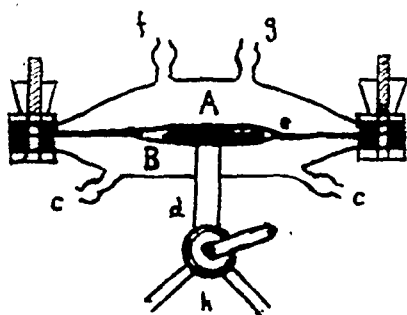


Fig. 4. Blood pressure regulator. *A*—upper chamber; *B*—lower chamber; *c*—inlet and outlet tubes for blood; *d*—overflow tube; *e*—rubber membrane with metal disc in center; *f*—tube connecting upper chamber to central stump of carotid artery; *g*—air outlet tube; *h*—stopcock.

The *pulse-frequency regulator* (*B* in fig. 3) is an electromagnetic valve, which clamps down on soft rubber tubes with every diastole and releases them on systole. This valve is activated through a U-tube, partly filled with mercury. A fine capillary tube connects its arms in order to equalise pressures when there is a change in the animal's blood pressure. This valve is activated by the animal's pulse or by a mechanical interrupter.

The *oxygenator* (fig. 5) is built on the principle of a Barcroft tonometer. Its main parts are two rotating cylinders of glass or tin-plated brass, one inside the other. The inner cylinder increases the oxygenating surface and distributes the blood in a thin uniform layer.

The instrument is held in position at a slant of about 25 degrees in a rocking joint at its upper end and by a rod turning freely in a tubular holder at the lower end. It is revolved at about 40–50 r.p.m. by a friction wheel connected to a flexible shaft. This oxygenator which is 50 cm. long and has a diameter of 6 cm. is capable of introducing 5 ccm. of oxygen per minute into 100 cc. of venous blood flowing at a rate of about 200 ccm. per minute. It may be built in different sizes according to requirements.

*The photoelectric apparatus for continuous measurement of the oxygen content of the blood is a modification of the apparatus described by Kramer and Winton*

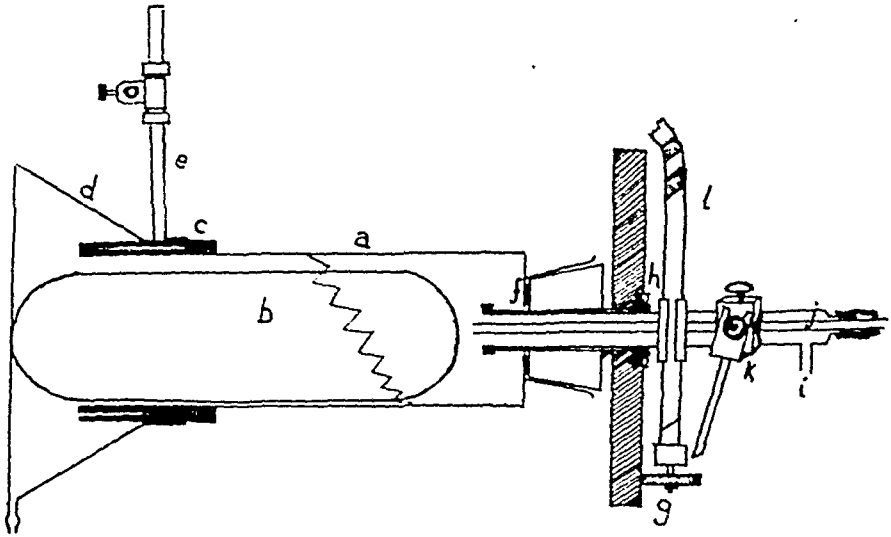


Fig. 5. Oxygenator. *a*—outer cylinder; *b*—inner cylinder; *c*—bearing; *d*—collecting funnel; *e*—swivel joint holder; *f*—rubber packing; *g*—friction wheels; *h*—ball bearing; *i*—gas inlet tube; *j*—blood inlet tube; *k*—rocking joint; *l*—flexible shaft.

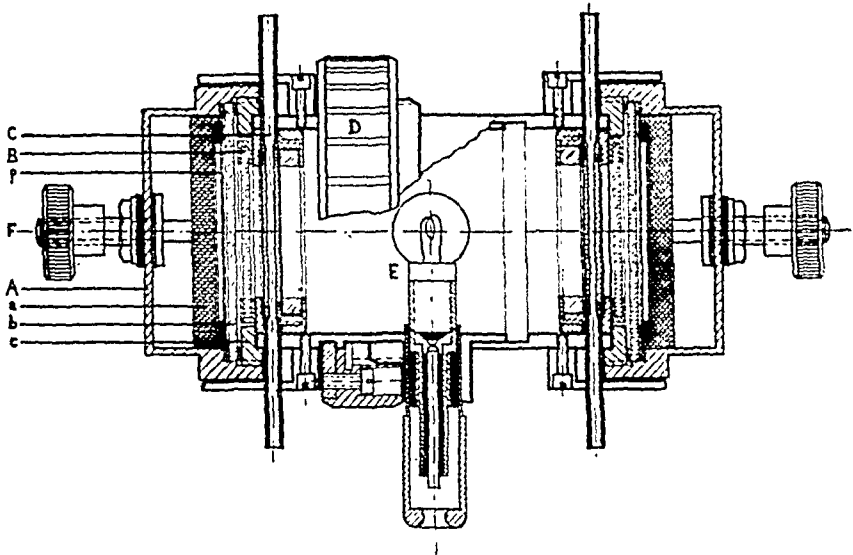


Fig. 6. Photoelectric apparatus for measuring A-V oxygen differences. *A*—metal housing containing photocell; *a*—hard rubber backplate holding photocell *P* and contacts; *b*—glass plate between rubber gaskets; *c*—threaded metal ring sealing photocell unit; *B*—red filter; *C*—cuvette; *D*—large adjusting screw for regulating distance of second photocell from light; *E*—6 volt, 0.5 amp. electric bulb in adjustable holder; *F*—photocell terminal.

(1939) and is constructed so that continuous and direct determination of arterio-venous oxygen differences can be made (fig. 6).

This apparatus makes possible the measurement of the respiratory metabolism of the brain even under rapidly changing conditions and dispenses with a large

number of blood gas determinations. It consists of two symmetrical halves, each containing a barrier layer photocell, a red filter (6200–6800 Å) and a new type cuvette through which the arterial and venous blood flow. The cuvettes are constructed so that the thickness of the central plate ( $d$  in fig. 7), which determines the thickness of the blood layer in front of the photocells, is identical in both halves. Thus the arterio-venous oxygen differences may be measured directly by connecting both photocells in compensation. Dismantling of the cuvettes, cleaning and reassembling can be carried out without changes in their thickness. The area of the slit in the central plate of the cuvette determines the area of illumination of the photocell. The flow velocity of the blood through the cuvette should be high enough to prevent sedimentation of the blood corpuscles which increases light transmission through the blood and may cause errors up to several hundred per cent. This can be prevented by a proper choice

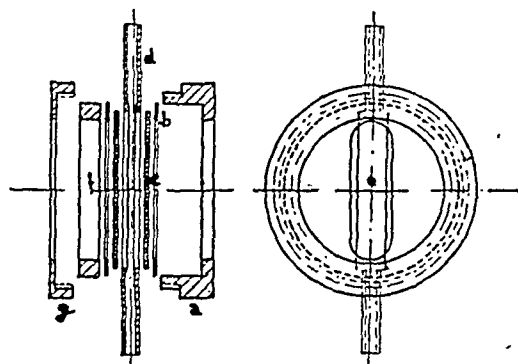


Fig. 7. Blood cuvette of photoelectric apparatus. *a*—metal housing; *b*—rubber gaskets; *c*—circular glass windows; *d*—central plate ground plane-parallel, 1.2 mm. thick; *e*—6 mm. wide slit in central plate through which blood flows; *f*—metal compression ring; *g*—threaded sealing ring.

of the dimensions of the slit in the central plate. In our apparatus the slit is 6 mm. wide and the central plate 1.2 mm. thick. Although the sedimentation rate of cat blood corpuscles is very high, measurements of arterio-venous oxygen differences with this apparatus were still reliable at a flow rate as slow as 5 ml. per minute. In order to translate galvanometer deflection into A-V-O<sub>2</sub> differences calibration curves were constructed using blood samples of different hemoglobin content. The measurements were made with the photocells connected in compensation and with the light, galvanometer and scale arranged so that a deflection of about 50 mm. corresponded to an A-V oxygen difference of 1 vol. per cent. It was found that by adjusting the light intensity so as to give the same galvanometer deflection (in our experiment 600 mm.) with every arterial sample, the same calibration curve could be used for any blood sample, having an oxygen capacity between 12–16 vol. per cent.

*The liver perfusion circuit for removal of vasoconstrictor substances from the blood* consists of a roller pump which conveys the blood from a large reservoir into the liver and back. The perfusion pressure is regulated by the height of an overflow vessel above the liver. About 2 hours before starting brain perfusion

the liver of a freshly killed cat which has been given a small amount of heparin and bled to death under ether, is placed in the apparatus with a cannula in the portal vein. The hepatic artery is tied and the hepatic veins are cut so as to allow free drainage from the liver. After washing out the liver with saline, perfusion is started through the portal vein with well filtered defibrinated ox blood to which a small amount of heparin has been added. The blood is passed through the liver at least 4 times after which it is transferred to the main perfusion apparatus and saturated with the desired gas mixture. During brain perfusion the blood does not come into contact with the liver.

The minimum amount of blood required in order to take maximum advantage of a perfusion experiment is about 600 ml. This includes about 250 ml. for analysis in an experiment of long duration, 150 ml. for one filling of the perfusion apparatus and a reserve of 200 ml. To have this amount of cat blood on hand would mean bleeding about 6 additional cats for each experiment. Consequently after a number of trials with defibrinated beef blood from the slaughter house, it became apparent that this could serve as well. The blood is usually collected the day before the experiment, filtered well through cloth and kept in a refrigerator. When kept for longer than 24 hours, Merthiolate-Na (Lilly) 1:20,000 is used as a preservative. We have never had any reactions from the use of bovine blood in the cat. Ox blood with its original high red blood corpuscle content offers excessive resistance to flow through the brain. Accordingly, the blood used for perfusion was diluted with plasma to give an  $O_2$  capacity around 14 vol. per cent. We have seldom found a higher oxygen capacity in the blood of narcotised cats and very often it has been even as low as 8 vol. per cent.

In our early experiments the blood was perfused before and during brain perfusion through the lungs of a freshly killed cat in order to get rid of the vasoconstrictor substances which develop in the shed blood upon standing. The very temporary effect of this procedure and the rapidity with which the lungs themselves became edematous, necessitated a search for a more efficient means of removing these v.c. substances. We found that by passing the blood several times through an isolated liver, it is possible to maintain a good blood flow through the brain indefinitely. In passing the liver, the blood will take up certain substances, thus the likelihood remains, that in addition to removing v.c. substances from the blood, the liver may in other ways be a vital factor in determining the success of brain perfusion.

*Analytical methods.* During experiments A-V oxygen differences are recorded continuously by the photoelectric apparatus described above. Samples for glucose, lactic acid  $CO_2$  and pH are taken from the arterial and venous sides of the perfusion system. Glucose is determined according to Somogyi (1937), lactic acid according to Friedemann and Graeser (1933),  $CO_2$  with the Van Slyke method and pH with the glass electrode.

**EXPERIMENTAL RESULTS.** The metabolism of the living cat's brain was recorded continuously while perfusing it with defibrinated heparinized ox blood. In some cases it was possible to keep the animal in excellent condition for over

two hours. At the start of perfusion a large part of the narcotic is washed out of the brain by the perfusing blood, the first 50–70 ml. of which are discarded. The small amount of narcotic remaining in the brain is greatly diluted by the perfusing blood. Thus, a few minutes after switching over the blood circulation of the brain to the perfusion apparatus, the animal seems to regain consciousness, showing perception of objects approaching its eyes, very active blinking reactions to light, rapid pupillary and corneal reflexes and spontaneous movements of the eyes and facial muscles. Respiration is good though rather fast, vasomotor and respiratory reflexes are very active and systemic blood pressure is fairly high throughout the good part of the experiments. At the same time the rate of blood flow through the brain and the oxygen consumption are comparatively high. On the other hand spinal reflexes remain slow as the cord is still under the influence of the narcotic.

*Oxygen consumption of the brain.* For convenience of presentation we divided the experiments into three groups according to the functional state of the brain and the behaviour of the animal during perfusion.

A. The first group comprises the best experiments in which cerebral activity was excellent and the animal seemed to be conscious as described at the beginning of this chapter.

B. The second group consists of those experiments in which spontaneous movements and other signs of near-consciousness were absent but ocular responses to touch and sometimes to light, and vasomotor and respiratory reflexes were good. As the pupils were constricted in most experiments of this group, the pupillary reflex could not be elicited.

C. To the third group belong those experiments or experimental periods in which reflex responses were very weak or absent. There was no spontaneous respiration and the systemic blood pressure was very low. The eyeballs protruded and were glassy and the pupils were extremely dilated.

In table 1 the oxygen consumption in each of these groups is shown, each experiment being divided into fifteen minute periods. The entire course of a typical experiment belonging to group A is shown in figure 8. In this experiment cerebral activity was excellent and the animal seemed to be conscious. The blood flow through the brain was high from the beginning of perfusion, well over 100 ml. per 100 grams brain per minute, with a perfusion pressure of about 120 mm.Hg. In this experiment as in most of this group, after the first 4–6 minutes of perfusion, blood flow rate through the brain increased spontaneously, perfusion pressure remaining constant, reaching rates up to 150 ml. per 100 grams per minute.<sup>2</sup>

The oxygen consumption of the brain was very high at the beginning of perfusion, sometimes 6 ml. of oxygen per minute. This high oxidation rate declined within 5 to 10 minutes, as shown in table 1, to a steadier level of oxidation between 4 to 5 ml. of oxygen. Oxygen consumption was maintained at a level usually over 4 ml. as long as cerebral activity remained excellent, in some cases

<sup>2</sup> Subsequently the figures for blood flow and for oxygen consumption are calculated for 100 grams brain per minute.

TABLE 1  
The Oxygen consumption of the perfused brain (ml. O<sub>2</sub> consumed per 100 grams brains per min.). Division into 3 groups according to functional state of brain

EXPERIMENT	DURATION OF PERFUSION, MINUTES												REMARKS	
	0-15	15-30	30-45	45-60	60-75	75-90	90-105	105-120	120-135	135-150	150-165	165-180		180-195
Group A														
23-5	4.65	4.65	4.50	4.80	4.65	4.42	4.35	3.78(1)	3.45	3.15(2)	3.22	3.34	3.34	(1) Added 60 mgm. % chloral hydrate. (2) Changed blood
30-5	4.75	4.23	3.60	3.30	3.08	3.22	3.15	3.00(1)	2.70	2.48	2.85*			(1) Changed blood—strong vasoconstriction
6-6	4.57	3.98	3.74	3.45(1)	3.08*	2.80								(1) Changed blood—vasoconstriction
13-6	5.45	4.20	4.12	4.42	3.90*	2.70								(1) Added 20 mgm. % Na-amytal
8-7	5.55	5.06	5.32	4.95	4.70	3.90(1)	3.00	2.85*						(1) Changed blood. (2) Added 20
15-7	4.73	4.50	4.87	4.75	4.65	4.42(1)	4.42	3.75(2)	3.00	2.85*				mgm. % Na-amytal
Average...	4.95	4.44	4.36	4.28		4.01								
Group B														
30-1	3.68	3.75	3.38*	3.15										(1) Added 20 mgm. % Na-pentothal
4-2	4.27	3.45	3.67	3.67*	3.45									
7-2	4.35	4.10	3.00	2.93*	2.55	2.40								
17-2	3.82	3.30	3.15*	3.15										
25-3	3.45	3.52	3.00	3.00*	2.65	2.40								
17-6	3.45	3.38	3.45*	3.22(1)	2.92									
Average...	3.83	3.58	3.27	3.18	2.90									
Group C														
25-2	3.22*	2.93	2.78	2.70	2.70									
28-2	2.55	2.40	2.10*	2.03	2.10									
4-3	3.00*	3.15	2.82	3.15										
11-3	2.32*	2.32	2.22	2.22	2.10									
21-3	3.75*	3.00	2.92	2.63	2.40									
3-7	3.75*	2.81	2.64	2.60										
Average...	3.10	2.77	2.58	2.56	2.32									

\* Loss of reflexes.

for periods up to 2 hours. Subsequently oxygen consumption slowly declined together with a progressive deterioration of cerebral activity. The experiments

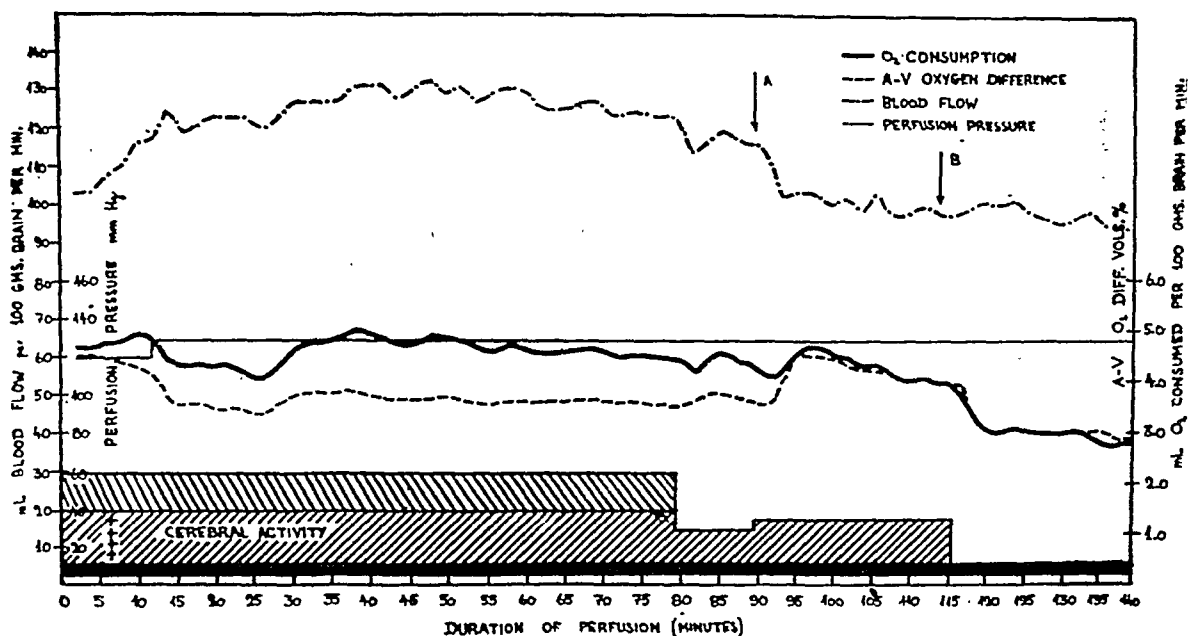


Fig. 8. Perfusion experiment with excellent cerebral activity (group A). At the bottom of the figure the uppermost crosshatched area indicates persistence of cortical activity, i.e., apparent consciousness, spontaneous movements, etc. The opposite crosshatching below it shows degree of reflex response as scored by four crosses. The thick black line above the abscissa shows the persistence of natural respiration. At A the perfusion blood was changed. At B 20 mgm. of Na-amytal were added to each 100 cc. of perfusion blood.

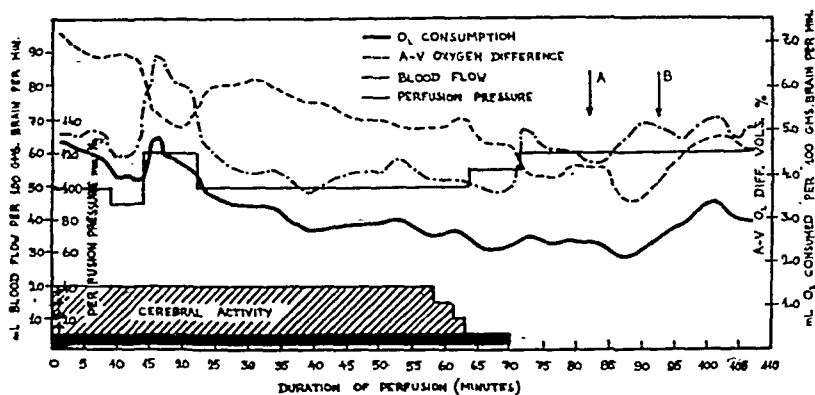


Fig. 9. Perfusion experiment with impaired cerebral activity (groups B and C), cortical activity absent. The first half of this experiment up to about 55 minutes is classified as belonging to group B, the latter part to group C. The duration of natural respiration is shown by the thick black line above the abscissa. At A 2 cc. of a 30 per cent NaCl solution were added to each 100 cc. of perfusion blood. At B 3 cc. of neutral M/5 phosphate buffer were added to each 100 cc. blood.

of this group were usually discontinued shortly after the disappearance of spontaneous activity. From numerous observations made on animals during perfusion experiments we feel justified in ascribing the observed high rate of oxygen

consumption in the experimental group A to cortical activity. In group B signs of cortical activity were absent though reflex activity was excellent and correspondingly a rate of oxygen consumption between 3.2-3.8 ml. was found.

There is a quantitative difference in their respective responses to narcotics between the cats in groups A and B. The addition of 20 mgm. of amytal per 100 ml. of blood abolished every sign of cortical activity spontaneous movements and ocular reflexes in an experiment belonging to group A though leaving natural respiration intact. At the same time the rate of oxygen consumption

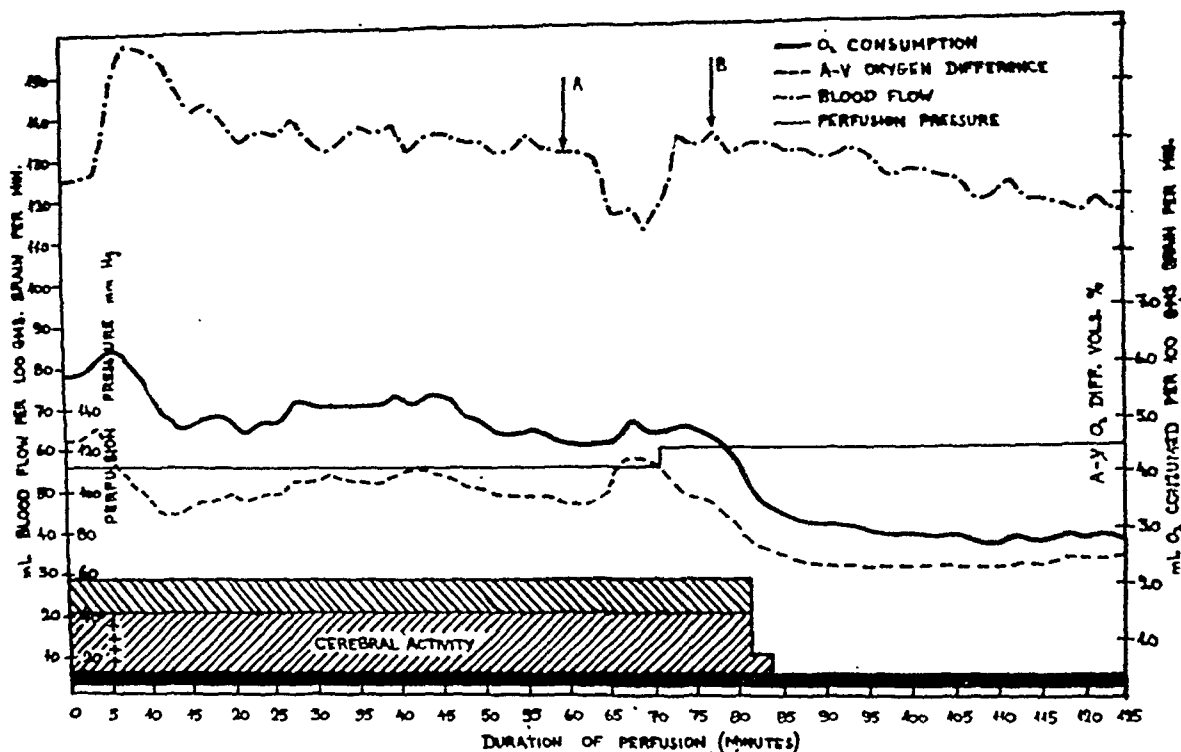


Fig. 10. The effect of narcosis on the oxygen consumption of the brain in an experiment with excellent cerebral activity (group A). Upper crosshatching shows cortical activity, thick black line natural respiration. At A blood was changed, at B 24 mgm. of Na-amytal were added to each 100 cc. of blood.

dropped from 5.1 to 2.9 ml. (43 per cent) as shown in figure 10. On the other hand the addition of 24 mgm. of amytal to the perfusion blood of a cat without cortical activity caused a reduction of oxygen consumption from 3 ml. oxygen to 2.5 ml. (17 per cent) only, as shown in figure 11.

*Hyperexcitability of the cortex* during strychnine and metrazol convulsions was always accompanied by a sudden very great rise of the oxygen consumption by the brain which set in together with the commencement of convulsions. The addition of barbiturates at once reduced the oxygen consumption to the previous level. Two experiments are shown in figures 11 and 12, one with strychnine, the other with metrazol. The addition of these drugs in concentration insufficient to cause convulsions or, when owing to the state of the brain convulsions could not be obtained, did not alter the rate of oxygen consumption.

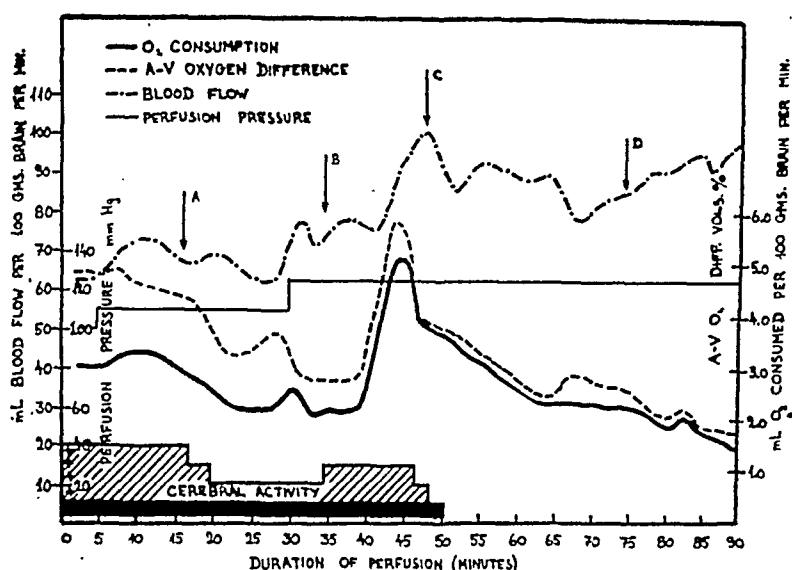


Fig. 11. Experiment, group B. At A 24 mgm. of Na-amytal, at B 2 mgm. of strychnine nitrate were added to each 100 cc. of blood. Convulsions occurred between the 38th and 48th minutes. At C the perfusion blood was changed. At D 22 mgm. of Na-amytal were added to each 100 cc. blood after all reflex activity had disappeared (group C).

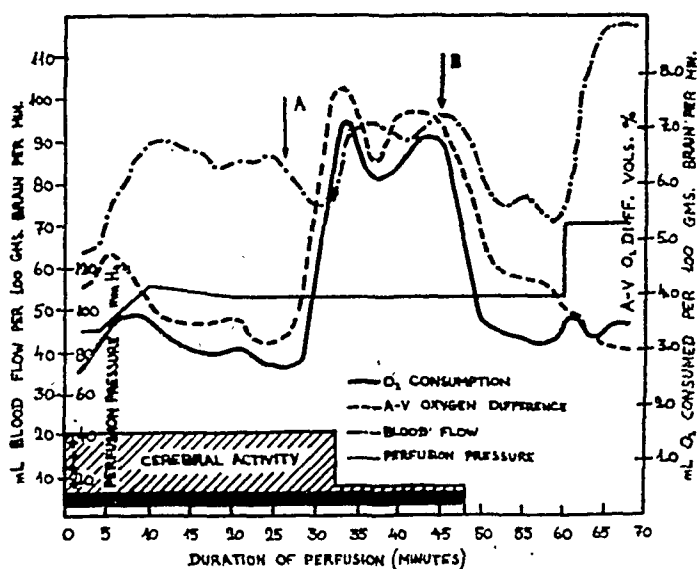


Fig. 12. Metrazol convulsions and their abolishment by pentothal (exper. group B). At A 60 mgm. of metrazol were added to each 100 cc. of blood. Convulsions occurred between the 28th and 48th minutes. At B 10 mgm. of Na-pentothal<sup>3</sup> were added to each 100 cc. of blood.

The rate of blood flow is an important factor in determining the oxygen consumption of the perfused brain. When the flow rate is high, between 100 to 150 ml. per minute (per 100 grams brain), increasing it affects oxygen consumption

<sup>3</sup> We are indebted to The Abbott Laboratories, North Chicago, Ill. for a generous supply of Pentothal Sodium.

but slightly. There is, however, a decrease in the oxygen consumption with decreasing flow rate even when the flow rate remains high. This is illustrated in figure 13. On the other hand, when the flow rate is below 80 to 90 ml. the oxygen consumption of the brain changes with every change in the rate of blood flow. In order to obtain maximum oxygen consumption, the flow rate has to be kept higher than 90 ml. per minute per hundred grams brain. Below this rate the blood flow is a factor determining the rate of oxygen consumption of the brain. Schmidt, Kety and Pennes (1945) measuring the blood flow and oxygen consumption of the monkey's brain also found that flow rate greatly influenced

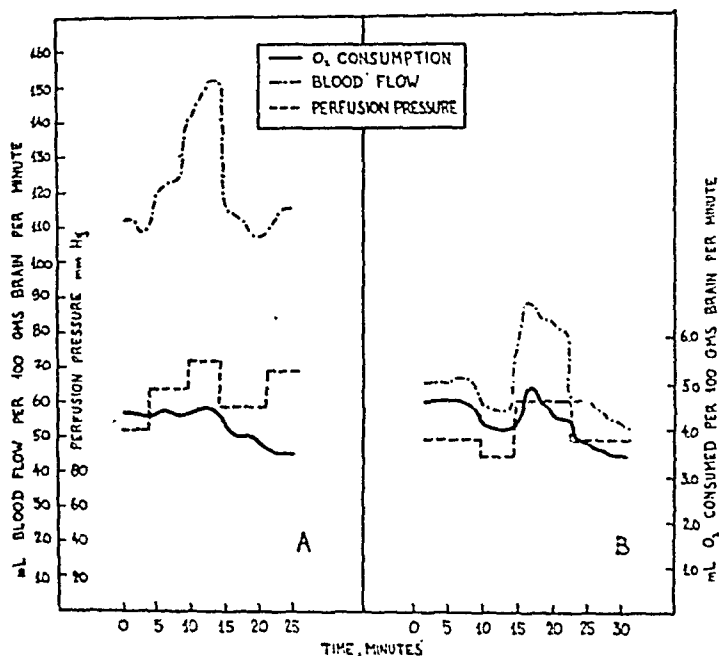


Fig. 13. Effect of changes in blood flow on oxygen consumption. A—at a high flow rate B—at a low flow rate.

its oxygen consumption. In their experiments the flow rates varied between 33 to 72 ml. per 100 grams brain per minute.

In all our best experiments after varying periods of perfusion, the oxygen consumption dropped and brain activity deteriorated. The addition of the known activators and substrates of brain carbohydrate metabolism has so far proved ineffective in restoring brain activity and oxygen consumption. A slight improvement of short duration could be obtained by raising the perfusion pressure. On the other hand the addition of fresh blood brings about a considerable increase in oxygen consumption together with improved brain activity, which improvement, unfortunately, is of short duration due to a simultaneous decrease in blood flow rate caused by the vasoconstrictor substances present in the freshly added blood. The slow deterioration of brain functions after a long period of perfusion with the same blood, and the improvement caused by the addition of fresh blood, seem to indicate that some substance which is indis-

pensable for the maintenance of normal brain functions is used up during perfusion.

*The effect of CO<sub>2</sub> tension*, of the addition of NaHCO<sub>3</sub> or of lactic acid was investigated in a large number of experiments. It appears that as far as these substances have any effect on the oxygen consumption of the brain, they exert it by altering the rate of blood flow. Thus, their effect on oxygen consumption was always marked in experiments in which the flow rate was originally low. A rise in flow rate is obtained by the addition of lactic acid, a decrease by the addition of sodium bicarbonate.

TABLE 2  
*The glucose consumption of the perfused brain*

EXPERIMENT	DURATION	GLUCOSE		PER 100 GRAMS BRAIN PER MINUTE		% GLUCOSE ACCOUNTED FOR BY OXIDATION
		Mgm.% in blood at start	Mgm. uptake by brain	Glucose mgm.	Oxygen ml.	
	<i>minutes</i>					
1	33	131	41.7	4.85	3.40	95
2	25	154	34.0	4.95	2.88	78
3	33	174	72.2	8.00	3.50	58
4	16	182	17.8	3.90	3.60	123
5	28	192	61.4	7.80	3.10	53
6	18	210	21.1	5.10	3.40	89
7	22	192	28.8	5.70	2.60	61
8	42	206	102.5	8.70	4.65	71
9	86	149	149.0	6.60	3.56	72
10	55	181	85.8	5.70	4.12	96.5
11	38	217	53.2	5.10	3.60	94
12	25	181	28.0	4.08	4.12	34

A considerable rise in systemic pulse pressure was frequently noted when the H ion concentration of the blood perfusing the brain was slightly increased by raising the CO<sub>2</sub> tension or by the addition of lactic acid.

*The diffusion of glucose into the brain.* The uptake of glucose by the brain was studied in 23 perfusion experiments. Flow velocity and oxygen consumption were registered continuously, glucose, lactic acid and pH were determined in arterial and venous samples. Analyses were made in duplicate. Special care was taken not to change the rate of venous blood flow while drawing the samples.

The glucose uptake for a given period was calculated from the change in glucose content of the reservoir and from the arterio-venous differences which have been estimated at frequent intervals. The maximum possible glucose consumption was calculated from the amount of oxygen consumed and from the lactic acid produced, if any. Comparing the figures of the amount of glucose which disappeared with the amount of oxygen consumed (usually lactic acid was not produced), it was found in most experiments that various amounts of glucose were unaccounted for. These experiments are shown in table 2.

We cannot give at present any explanation for the discrepancies between glucose disappearance and oxygen consumption as the periods under investigation were in most cases not long enough to exclude the effect of possible fluctuations in the diffusion rate. On the other hand, frequent determinations of arterio-venous glucose differences have shown that such fluctuations exist and are especially marked when the glucose content of the perfusing blood is being changed. Figure 14 shows such an experiment in which the rate of the entrance of glucose fluctuates although the oxygen consumption remains unchanged at the same time. If the glucose content of the perfusing blood is higher than that of the

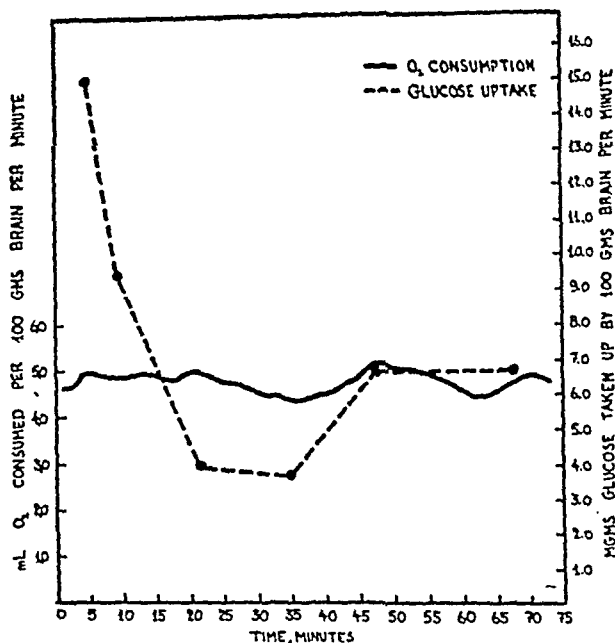


Fig. 14. Fluctuations in the glucose uptake of the brain without corresponding changes in oxygen consumption. The ordinates are so arranged that each milligram of glucose uptake (righthand ordinate) corresponds to 0.75 mL of oxygen consumed (lefthand ordinate).

cat's, the glucose uptake at the beginning of perfusion is higher than the corresponding oxygen consumption. This also happens after the addition of glucose to the perfusing blood. Conversely the venous blood may even contain more glucose than the arterial when the glucose concentration in the perfusing blood is suddenly lowered by changing the blood to one of low glucose content. In this latter case glucose is leaving the brain in order to establish a new osmotic equilibrium with the perfusing blood. From the foregoing it is obvious that the rate of glucose uptake is not identical with the rate of glucose oxidation but represents the sum of the glucose actually metabolised and that necessary to establish osmotic equilibrium.

In the hope of being able to measure the diffusion rate of glucose into the brain, several experiments were made in which a certain amount of glucose was added to the blood and, at very frequent intervals, (2 to 4 min.) arterio-venous samples

were taken for glucose determination. In these experiments a rather striking fact came to light. Only into the dead or deeply narcotized brain does glucose penetrate according to a regular and uniform pattern as shown in figure 15.

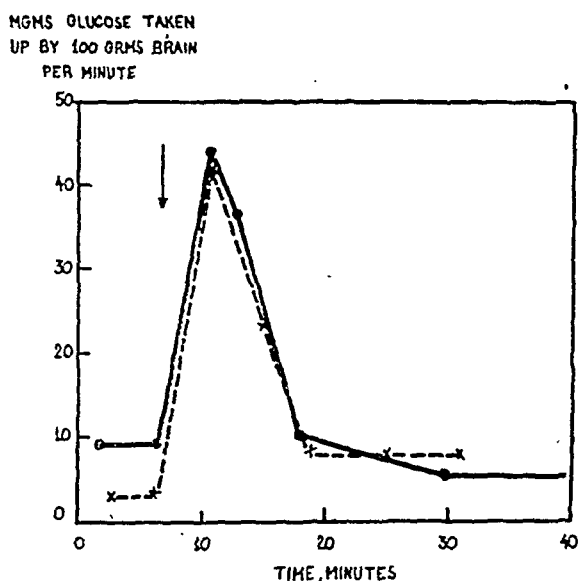


Fig. 15. The course of glucose uptake by the deeply narcotized brain (continuous line) and by the dead brain (broken line). At the arrow 100 mgm. of glucose were added to each 100 cc. of blood. The experiment with the deeply narcotized brain (continuous line) is a continuation of the experiment represented by the continuous line in figure 14, but after 20 mgm. of Na-amytal had been added to each 100 cc. of blood.

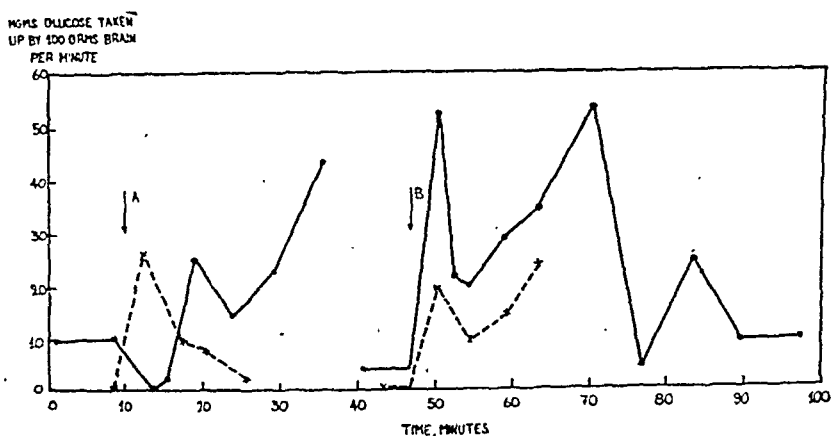


Fig. 16. The course of glucose uptake by the active brain, shown in two experiments (continuous and broken lines), after the addition of 100 mgm. of glucose to each 100 cc. of perfusion blood at A and at B.

In these experiments right after the addition of glucose there is a high rate of glucose uptake into the brain which reaches a peak during the first four to five minutes and then declines within ten to fifteen minutes to a fairly constant level. On the other hand, the diffusion of glucose into the active brain is irregular and

individual as shown in figure 16. These experiments which were made on cats with excellent cerebral activity have shown large irregular oscillations in glucose uptake while the oxygen consumption remained constant. As these oscillations did not occur in the dead or deeply narcotised cat, the assumption is made that the permeability of the brain to glucose is connected with its functional activity. This point is further illustrated by experiments on one and the same animal in which the diffusion of glucose into the brain was measured while brain activity was excellent and again after spontaneous and reflex activity have been abolished by sodium amytal. In figure 16 the glucose uptake after the first addition is very irregular, while after the addition of the narcotic it corresponds with that of the dead brain.

#### SUMMARY

1. The anatomical details and surgical difficulties involved in isolating the cerebral circulation are described. The venous outlets from the brain of the cat, particularly the sinusii columnae vertebralis and an important communicating branch between the vertebral and the external jugular veins are described.

2. A new method for the isolation of the cerebral circulation is described. It consists in occluding all venous outlets from the brain, including the sinusii columnae vertebralis with special clips, the ligation of certain arteries, and in tapping two occipital sinuses for the collection of the cerebral venous blood in toto.

3. A very effective method for the removal of vasoconstrictor substances from the blood by perfusing it through an isolated liver is described.

4. A perfusion apparatus, especially adapted for brain perfusion, is described in detail. Some of its features are:

- a. A pressure regulator which is under central vasomotor control.
- b. A pulse-frequency regulator also under central vasomotor control.
- c. An efficient new-type blood oxygenator.
- d. A cuvette for a photoelectric apparatus for direct continuous measurements of arterio-venous oxygen differences in flowing blood.

5. Experiments are described in which cats were kept alive with apparently good cerebral functions while perfusing the brain with defibrinated heparinized ox blood. In the best experiments cerebral functions were maintained in excellent condition for perfusion periods of two hours and more as manifested by near-consciousness, spontaneous movements, natural respiration and very active ocular, respiratory and vasomotor reflexes. During these experiments continuous records of cerebral blood flow and of A-V oxygen differences were made. Arterial and venous blood samples were withdrawn at suitable intervals for analysis of glucose, lactic acid,  $\text{CO}_2$  and pH.

6. The oxygen consumption of the brain was found to be related to the degree of cerebral activity. Hence the experiments were divided into three groups according to the functional state of the brain.

In group A cerebral activity was excellent (spontaneous movements, etc.) and  $\text{O}_2$  consumption was at its highest (4-5 ml. per 100 grams per min.). In

group B with good reflex activity but without spontaneous movements, etc. the  $O_2$  consumption lay between 3.2–3.8 ml. per 100 grams brain per minute. In group C with very weak or no reflex activity,  $O_2$  consumption was the lowest (2–3 ml. per 100 grams per min.). The high rate of  $O_2$  consumption in group A is attributed to maintained cortical activity.

7. The effect of adding various barbiturates to the perfusion blood was studied. All of the substances used (amytal, luminal, dial and pentothal) depressed cerebral oxygen consumption and brain activity. When brain activity was excellent and oxygen consumption high (group A), the addition of a small amount of narcotic to the brain depressed oxygen consumption greatly and abolished all signs of cortical and reflex activity. When cerebral activity was only fair and oxygen consumption lower, adding the same dose of narcotic had a relatively much weaker depressant effect on oxygen consumption.

Amytal and luminal were without effect on cerebral blood flow whereas dial and pentothal had a vasoconstrictor effect. Administration of amytal to the perfusion blood was instrumental in raising the systemic blood pressure.

8. During metrazol and strychnine convulsions very high rates of cerebral oxygen consumption were observed and blood flow rate also rose. Convulsions could be abolished by the addition of barbiturates to the perfusion blood. When the convulsant drug was given in subconvulsant doses or to moribund animals, the oxygen consumption did not change.

9. The relation of cerebral blood flow to  $O_2$  consumption was analysed. When the blood flow was above a level of about 90 ml per 100 grams brain per minute, changes in the rate of flow had but little effect on  $O_2$  consumption. Below this level the  $O_2$  consumption varied with the rate of blood flow so that at low flow rates the rate of blood flow becomes an important factor in determining the rate of  $C_2$  consumption.

10. Contrary to expectations, none but extreme changes in the  $CO_2$  tension or other changes in the pH of the blood (addition of  $NaHCO_3$  or lactic acid) had any effect on cerebral blood flow. When there was an effect, increased pH lowered blood flow and increased acidity increased flow. Very often an increase in the H ion concentration of the perfusion blood caused a rise in the systemic pulse pressure of the cat and sometimes in the mean blood pressure as well.

11. The glucose uptake by the brain showed considerable variations when followed over fairly long periods of time, though the oxygen consumption remained constant. Frequently the amount of glucose disappearing was in excess of that which could be accounted for by oxygen consumption and lactic acid production.

Frequent determinations of A-V glucose differences showed fluctuations in the rate of glucose diffusion into the brain which were especially marked upon changing the concentration of glucose in the perfusing blood.

The glucose uptake of the brain represents the sum of the glucose actually metabolized and of that necessary to establish equilibrium between blood and brain.

The glucose uptake by the deeply narcotized or the dead brain, after the addition of glucose, followed a regular reproducible pattern. On the other hand diffusion into the active brain was very irregular and individual. It is suggested that the permeability of the brain to glucose is associated with its functional activity.

It is a pleasure to acknowledge the untiring help of Mr. Z. Tuttnauer, head of our mechanical workshops. To Mr. A. Sochazewer we are indebted for helpful suggestions in the construction of the apparatus. Finally we wish to thank Dr. S. Meiboom of The Department of Physics for his constant advice in connection with the construction of the photoelectric apparatus. The photoelectric apparatus and the clips for compression of the spinal venous sinuses were made by Mr. H. Kahn and the glass parts of the perfusion apparatus by Mr. Rosenbaum.

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# ELECTROMYOGRAPHIC INTERFERENCE IN THE HUMAN ELECTROENCEPHALOGRAM

## A STUDY OF THE EFFECT OF MILD CURARIZATION

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Interpretation of the electroencephalogram is made difficult by a variety of natural and man-made factors. Frequently the record of cortical activity is intermixed with spike potentials produced by cranial muscles. Although the spike is characteristically a fast disturbance, a single spike being only 0.01 second in duration, discharges of 5 to 50 times per second which are well within the range of EEG reception often occur. Gibbs (7) has stated that if the "cut-off" (i.e., recording with diminishing amplitude) is too low or if the "damping" of the instrument is too much, the slower components of muscle potentials stripped of their fast components closely resemble waves of cortical origin.<sup>1</sup> The detection and appraisal of muscle frequencies under these circumstances may be complicated.

Davis (4) noticed particularly that the frontal leads were distorted by electromyographic interference; and Gibbs (7), when working on infants, problem children, feeble-minded persons and agitated psychotics, frequently found it impossible to obtain a satisfactory frontal record. Finley (6), in a recent study, reported the occurrence of rapid-frequency potentials of moderate voltage, especially in agitated mental states, principally over the anterior portion of the cerebrum. At this point, electrodes for recording the cerebral activity of the corresponding lobes overlie muscle bellies of (a) the frontalis muscle which interdigitates with the procerus, orbicularis oculi and corrugator, and (b) the temporalis muscle which underlies the auriculares.

The order and magnitude of action potentials from these muscles as well as the degree and extent of their insinuation into the corticogram remain to be explored. Investigation ultimately might be expected to eliminate the confusion, frustration and retrials necessary in certain records such as those described by Davis (4): "... A formless pattern which looks like beta activity at greater amplification on a slower tape ... for 2 years this quality in the record was confused with muscle potentials ... one record on an individual may not be enough to determine whether a 'choppy' quality represents muscle potentials or whether it is due to a cerebral lesion. One must use bipolar as well as mono-

<sup>1</sup> The frequency of recording is flat from 3 to 65 cycles per second; the upper limit is often deliberately diminished by a "muscle filter" built into the power amplifier to 50 cycles per second for purposes of reducing extraneous electrical disturbances and muscle potentials to a minimum. Mechanical pressure of the inkwriters and variations of gain, attenuation and filter factors determine "damping".

polar recording, compare corresponding areas of the head and use every precaution to rule out artifacts or muscle potentials."

Of particular interest here was the fact that the drug curare is capable of blocking striated muscle while producing no cerebral effect discernible in the electroencephalogram. Action currents from curarized human muscle, as shown by studies with aqueous curare by Harvey and Masland (12), fall to 40 per cent of normal; weakness persists in variable degree for a period of 15 to 30 minutes after intra-venous injection, and for several hours after intramuscular injection. Also they noticed no electroencephalogram change in their studies. Reports of other work with an aqueous solution of curare have indicated that doses short of respiratory embarrassment produce no alteration in the human electroencephalogram (10). Using curarized dogs and monkeys completely paralyzed and maintained with artificial respiration, Girden (9) was also unable to demonstrate changes in the electroencephalogram. He deduced from this that anoxia of the cortex secondary to respiratory paralysis might be responsible for electroencephalogram abnormalities observed in frogs (5).

The purposes of this study were: 1, to determine whether the anterior cerebral leads are compacted of muscle and cortical potentials, and 2, to estimate the value of curare in distinguishing the two potentials.

**EXPERIMENTAL METHOD.** *Material.* Thirty-eight subjects provided the electroencephalograms and electromyograms that were employed in the study. Selection was virtually random. Ages varied from 19 to 39. Diagnostic classification was:

Craniocerebral trauma . . . . .	12
Psychosis . . . . .	7
Psychoneurosis . . . . .	6
Epilepsy, petit and grand mal . . . . .	2
Sciatic neuritis . . . . .	2
Undiagnosed . . . . .	2
No disease . . . . .	2
Migraine . . . . .	1
Hemichorea . . . . .	1
Diabetes insipidus . . . . .	1
Retinitis pigmentosa . . . . .	1
Peripheral facial palsy . . . . .	1
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	38

*Recording system.* All records were obtained on a four-channel, condenser-coupled Grass amplifier equipped with an ink writing oscillograph. Tape speed was 3 cm. per second. Sensitivity ranged from 15 mm. per 100 microvolts for the electroencephalogram to 2 mm. per 100 microvolts for the electromyogram. Adjustments of gain, attenuation and filter factors were varied individually in obtaining electromyograms. Settings were constant for all electroencephalograms. Both monopolar and bipolar recordings were used. Subjects were usually seated, but sometimes reclining. Fifteen minutes of activity recorded on each included three to five minutes of hyperventilation. An additional 15

minutes were added to the tracings, using special frontal and temporal leads. Muscle activity was simultaneously recorded with the electroencephalogram during "relaxation" or from "maximal frowns" by the patient every 30 to 60 seconds.

*Interpretation and classification.* Electroencephalograms were interpreted according to the present classification of Gibbs, Gibbs and Lennox (8).

*Electrodes.* Cortical potentials were obtained from the characteristic cranial placements using flat surface discs, 0.5 cm. in diameter, applied with electrode paste. Group muscle unit and single muscle unit records were electrically tapped by overlying surface discs, needle electrodes (size 26 or 27) or coaxial needles imbedded in the cranial muscles and connected to the input of the amplifier.

*Drug application.* A run of "normal" record was interrupted by the injection of an aqueous solution of curare<sup>2</sup> into the antecubital vein. Injection time was 60 seconds. The amount varied with body weight and was usually 0.5-1.0 mgm. per kilo. Dosage ranged from 30 mgm. to 80 mgm.

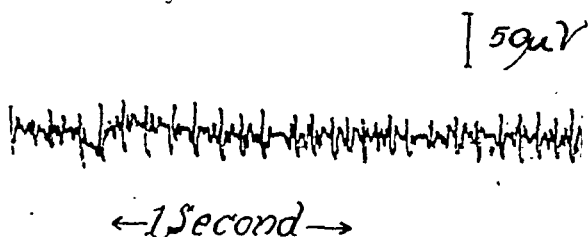


Fig. 1. Muscle units firing from the frontalis muscle. Recording with a coaxial needle electrode. Subject relaxed, eyes closed.

**RESULTS.** a. *Experimental.* During closure of the eyes under mild to moderate tension, discrete discharges arising from the frontalis muscle were demonstrable (fig. 1). With slight to moderate movements of the jaw similar discharges were found to be emitted by the temporalis muscle. In some "relaxed" individuals a mild ripple of electromyographic activity was constantly present, especially over the frontal region. At times this activity could be governed by placing the fingers gently on the eyelids (fig. 2).

An unusual demonstration was provided by the subject represented in figure 3. Here a prolonged muscle unit discharge occurred over the temporoparietal region during the course of a 16 electrode search. At the end of 7 minutes the activity was terminated within 60 seconds by 60 mgm. of curare intravenously. There was concurrent improvement in the fronto-precentral and postcentral-occipital leads. The subject was co-operative and "relaxed" during the entire recording.

In figure 4 a pronounced and more irregular disturbance of high-voltage fast spike activity in the electroencephalogram is demonstrated. The patient was a severe catatonic, only mildly co-operative, who shut his eyes tightly

<sup>2</sup> Solution of d-tubocurarine (Intocostin), courtesy of E. R. Squibb & Co.

and wiggled his ears intermittently. With mild curarization the fast activity decreased latest in both magnitude and frequency, from the frontal and temporal leads. Fast activity reappeared first over these areas when the effects of the drug were dissipated.

Because of difficulties in obtaining and controlling single muscle unit discharges from the scalp areas under investigation, a simple but less precise method of recording electromyograms was instituted by using two sewing needle tips 1 to 2 cm. apart inserted into the frontalis or temporalis muscles. With this method, combined electroencephalograms and electromyograms, from the frontal or temporal regions illustrated similar changes after curare injections. Simultaneous diminution of rapid-frequency potential waves was observed (fig. 5). The initial electroencephalogram in figure 5 was predominately low-to-moderate voltage, rapid-frequency in type.

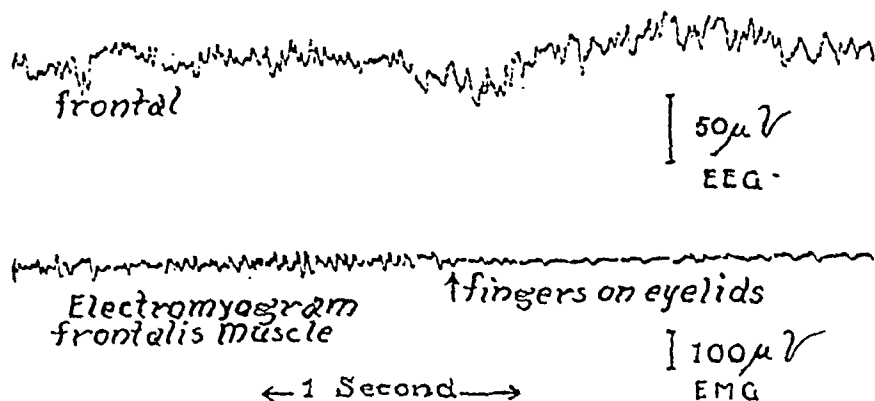


Fig. 2. Decline in number and amplitude of potentials in the frontalis electromyogram after placing fingers on closed eyelids.

Similar positive control studies with curare, on records showing mainly synchronous, sustained, 9 to 12 per second rhythm (alpha), exhibited no alteration in the electroencephalogram, but showed an invariable decrease in the frequency and voltage of the electromyograms taken simultaneously. In all trials the electromyogram was altered by curare.

The use of 3 cc. of distilled water intravenously in negatively controlled recordings resulted in no electromyographic or electroencephalographic change, despite either the varying frequency-spectrum predominant, or the previously observed decline in spike activity after curare (table 1).

In general, comparisons of simultaneous electromyograms and electroencephalograms recorded before and after partial curarization, disclosed a decrease in average amplitude and total oscillations of originally rapid-frequency potentials. The individual form of the electromyogram records was invariably preserved, while the electroencephalogram tracing was moderately altered, usually approaching the basic configuration of the occipital and parietal leads.

b. *Statistical.* Forty electroencephalogram trials were taken on 38 subjects; 37 subjects were treated with curare. Results are as charted in table 1.

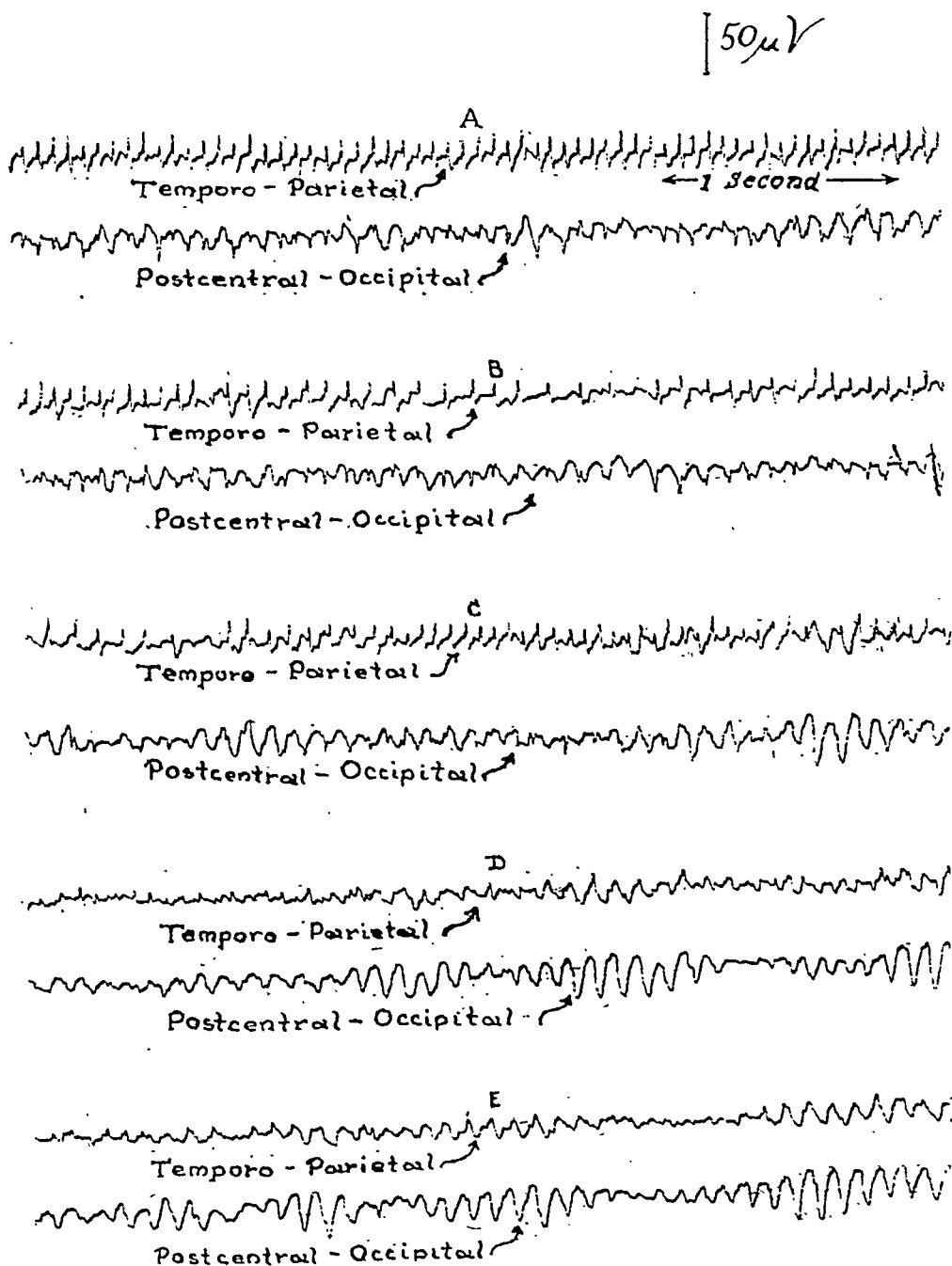


Fig. 3. Muscle unit activity emanating from the temporo-parietal region during electroencephalography. Obliteration of interference after 60 mgm. of curare intravenously. Male subject. Age 22. Weight 170 pounds. Diagnosis: Epilepsy, petit mal.

A. Prior to injection. B. 40 seconds after injection. C. 50 seconds after injection. D. 2 minutes after injection. E. 5 minutes after injection.

As judged by the decrease in number and amplitude of recognizable spikes in the combined anterior cerebral leads and electromyograms, 49 per cent of the trials showed a positive change after the test, 43 per cent no change, and 8 per

cent were indeterminate. In 16 (or 89 per cent) of 18 records exhibiting alterations with the drug the main frequency distribution in the electroencephalogram was over 12 cycles per second. In the group of 18 with demonstrable changes, 2 records (or 11 per cent) exhibited frequencies below 12 cycles per second.

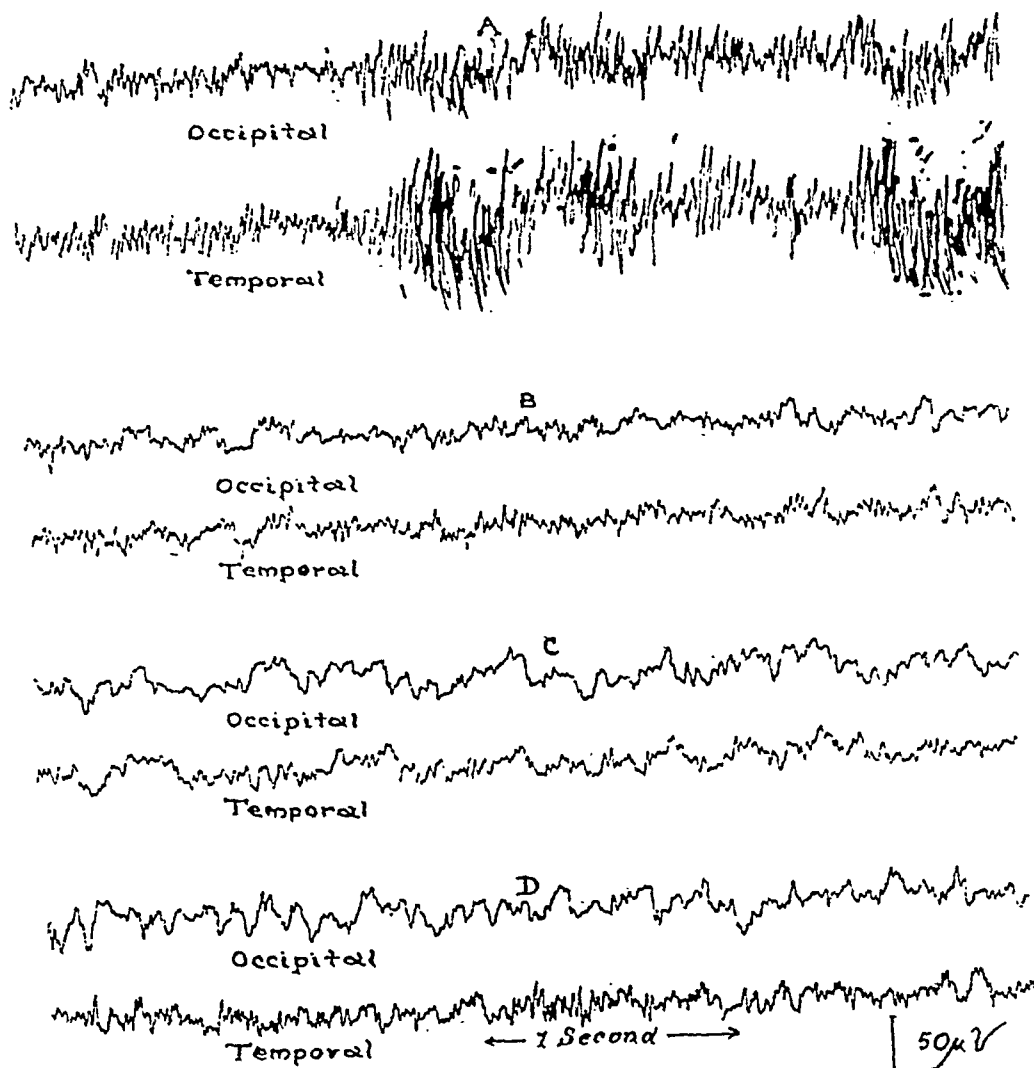


Fig. 4. Decline of interfering spike activity in the EEG after 40 mgm. of curare intravenously. Male subject. Age 21. Weight 132 pounds. Diagnosis: Schizophrenia, catatonic.

A. Prior to injection. B. 2 minutes 30 seconds after injection. C. 5 minutes after injection. D. 9 minutes after injection.

In the next series of 16 records exhibiting no change in the frequency-spectrum after curare, 8 (or 50 per cent) were over 12 cycles per second and 7 (or 44 per cent) showed a predominant frequency of 8.5 to 11.5 cycles per second.

Of the subjects in whom electroencephalographic interference was suspected during the routine run of the electroencephalograms, two-thirds subsequently

showed frequency changes after curare. Suspicion was based on the appearance of sporadic or diffuse rapid-frequency, moderate-voltage waves emanating especially from the frontal and temporal leads.

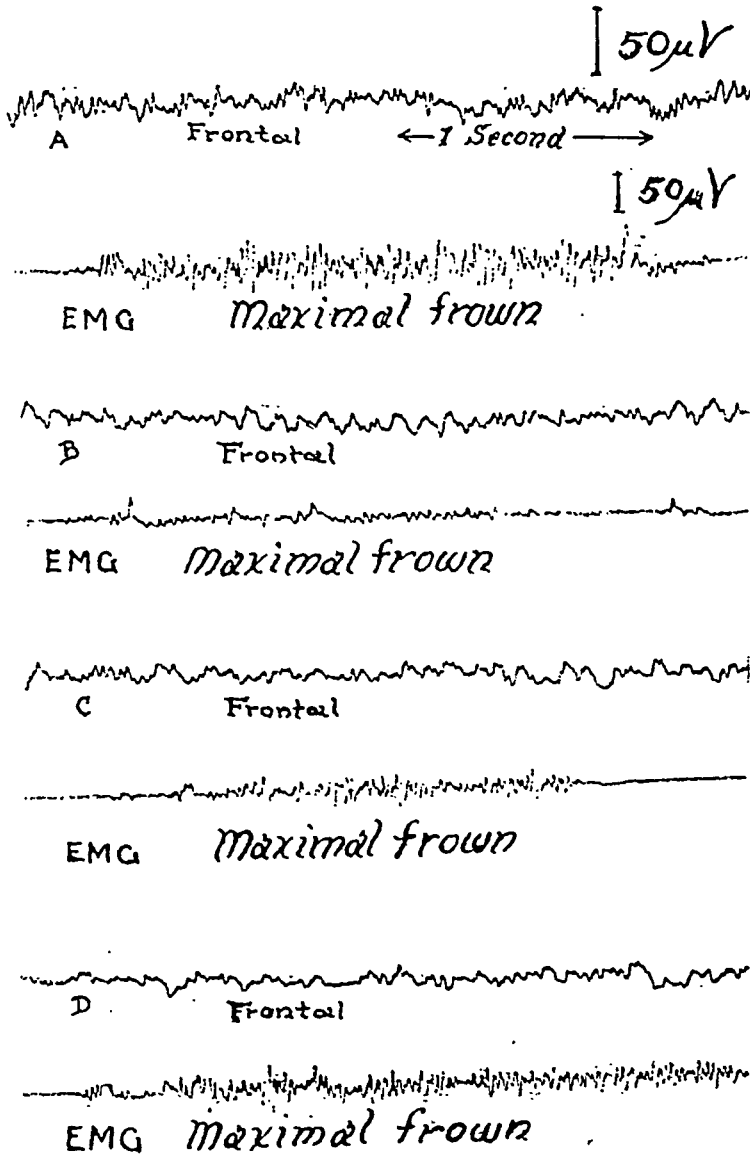


Fig. 5. A comparison of the spike potential decline in the frontal EEG and electromyogram after curarization with 55 mgm. intravenously. Female subject. Age 31. Weight 141 pounds. No disease.

A. Prior to injection. B. 3 minutes 30 seconds after injection. C. 8½ minutes after injection. D. 13 minutes after injection.

The incidence of records interpreted as abnormal was highest in the group with altered frequencies following curare; the interpretation of records as normal was highest in the unaffected group (table 1). These correlations were based on the electroencephalograms usually interpreted without regard for the outcome

TABLE 1

*Incidence of frequency decline in varying bands of the electroencephalogram spectrum following curarization*

MAIN FREQUENCY BAND	NO. SHOWING DECREASE IN FREQUENCY	NO CHANGE	INDETERMINATE	H <sub>2</sub> O CONTROL
Low voltage fast.....	11	6	0	0
F <sub>2</sub> .....	0	0	0	0
F <sub>1</sub> .....	5	2	0	1
8.5-12 cps.....	1	7	2	1
S <sub>1</sub> .....	0	1	0	1
S <sub>2</sub> .....	1	0	0	0
Unreadable.....	0	0	1	0
Total.....	18 (49%)	16 (43%)	3 (8%)	3
Suspected EMG Interference Prior to Test.....	8	4	1	1
Interpretation:				
Normal.....	4	11	2	1
Abnormal.....	10	2	0	1
Borderline.....	4	3	0	1

TABLE 2

*Diagnoses of subjects related to curare response*

	NO. SHOWING DECREASE IN FREQUENCY	NO CHANGE	INDETERMINATE	H <sub>2</sub> O CONTROL
Cranial cerebral trauma.....	5 (4)*	6 (3)	1	1 (1)
Psychosis.....	5 (5)	1 (1)	1 (1)	
Psychoneurosis.....	2 (1)	3 (1)	1	1 (1)
Epilepsy.....	1 (1)	1 (1)		
Sciatic neuritis.....		1		1
Undiagnosed.....	1 (1)	1 (1)		
No disease.....	2 (2)			
Migraine.....	1 (1)			
Hemichorea.....		1		
Diabetes insipidus.....		1		
Retinitis pigmentosa.....		1 (1)		
Bell's palsy.....	1 (1)			
Total.....	18	16	3	3

\* Parentheses contain number of records with main frequency over 12 cycles per second

of the curare studies. Since these studies were mainly applied to the frontal and temporal areas, no comprehensive statement can be made as to whether the interpretation of any record was altered as a result of the curare test.

When the diagnoses of the subjects tested were related to the changes with curarization (table 2), little correlation was noted, although a relatively high

number of psychotics (5 out of 7) showed rapid-frequency potentials (especially over the frontal and temporal regions), which were affected by the drug.

Counts of frequency differences based on counts of recognized spikes for tracings of both electromyograms and electroencephalograms prior to and following drug injections were compared with the time of maximum change with the drug. A general parallelism in oscillation change and temporal distribution was found. In both types of tracings a frequency decline of approximately 10 to 20 cycles per second occurred within  $\frac{1}{2}$  to  $3\frac{1}{2}$  minutes after the injection of curare.

Amplitude decrements varied more widely, in a range of 10 to 100 microvolts, representing variations from 50 per cent to 20 per cent of the original.

c. *Pharmacologic.* The effects of mild curarization with 0.5–1.0 mgm. per kilogram of curare were generally constant. The usual response noted was relaxation of the scalp, eyelids, face, and jaws; often there was mild blurring of vision. A few subjects described generalized relaxation or, rarely, weakness of the extremities. Transient labored respiration (20–30 sec.) was observed twice. One subject described no symptoms, although a marked decrease of electromyographic frequencies in his record was noted.

There was a parallel distribution of change in electroencephalogram and electromyogram records during the onset, peak and duration of drug effect. The onset of effect in most tracings was reached in 1 to 2 minutes, the peak in 3 to 5 minutes, and the duration lasted from 12 to 18 minutes.

DISCUSSION. Sizable muscle potentials, as described and recorded previously by many investigators (1) (2) (14), may also be obtained from the anterior cranial muscles. These potentials equal or may even exceed cortical potentials registered from the conventional anterior cranial electrode placements. From the standpoint of routine EEG technique, it is interesting that more tension is placed on the eyelids during closure (orbicularis, frontalis muscles contracting) with the jaw open than when shut (3); yet a closed jaw tightens the temporalis muscle.

Following partial curarization of some patients during the recording of electroencephalograms and electromyograms, there is a decline in amplitude of similar, discrete and diffuse, spike potentials visible in both tracings. In these studies comparisons of figures on recognizable spike potentials indicate a roughly parallel and proportionate decrease in number and amplitude following curarization. Although counts irrespective of size, differences in amplifier sensitivity and lack of measured contraction tensions prevent precise evaluation, the observations denote the possibility of considerable distortion of the corticogram by electromyographic interference. The interference has been found to be most prevalent over the anterior cranial muscles, and small doses of curare may readily obliterate or modify it. A similar decrease in spike amplitude has been described in muscle potential studies on myasthenia gravis patients (11) (13).

About one-half the number of random selected trials in this series disclosed the presence of electromyographic interference. In only one-tenth of this group was there no evidence of predominant rapid-frequency potentials over

the frontal and temporal leads of the EEG. The explanation for this becomes partially apparent when one considers that the statistics based on predominant frequencies would not include a mild amount of rapid deflections over the areas studied.

However, rapid-frequency potentials were observed in one-half of the 43 per cent of trials unchanged after curarization. It is to be remembered that although the electroencephalogram was unaltered, the electromyogram recorded simultaneously was invariably altered and usually clinical symptoms of curarization were observed. Re-examination of the protocols in the eight trials representing this group of rapid-frequency potentials unaffected by curare revealed that two subjects received an insufficient dosage and that two had mild toxic symptoms (transient labored respirations) nullifying the observations.

It is significant that two-thirds of the records exhibiting rapid oscillations, and two-thirds of the records suspected of electromyographic interference prior to the trials, were of the group affected by the drug.

The prevalence of electroencephalograms interpreted as abnormal where electromyographic interference was demonstrated is of uncertain significance. The necessity for greater study of rapid-potentials in relation to intracranial disturbances suggests itself.

#### CONCLUSIONS

1. Electromyograms on relaxed subjects during the course of electroencephalography indicate the presence of tension in the anterior cranial muscles.

2. In some subjects, rapid deflections of similar form from the frontal and temporal leads in simultaneous electroencephalograms and electromyograms show a parallel decline in number and amplitude after partial curarization, denoting electromyographic interference in the electroencephalogram.

3. Insinuation of myopotentials into the electroencephalogram usually takes the form of the mass or multiple unit electromyogram representing varying degrees of unit summation and asynchronous discharge.

4. Of a random selected group of patients, electromyographic interference in the electroencephalogram has been found in approximately 49 per cent. Of this number, 89 per cent exhibited rapid frequency potentials of moderate-to-high amplitude, mainly over the anterior cerebral leads. Of all the records demonstrating chiefly rapid-frequency potentials over the areas studied, two-thirds exhibited a response to curarization.

5. The use of curare intravenously in dosages of 0.5 to 1.0 mgm. per kilogram may be found helpful as an adjunct in the interpretation of electroencephalograms.

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# REGENERATION RATES OF SERUM CHOLINESTERASE IN NORMAL INDIVIDUALS AND IN PATIENTS WITH LIVER DAMAGE<sup>1</sup>

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Serum esterase activity results from the ability of one or more enzymatic proteins of the serum to catalyze the hydrolysis of certain choline esters. Brauer and Root (1) have shown in rats that liver damage induced by carbon tetrachloride results in a depression of plasma cholinesterase activity; this they attribute to a diminished synthesis of the enzyme by the damaged liver. The absence of any cholinesterase-inhibiting substance produced by the experimentally damaged liver is evidenced by the lack of any dilution effect on enzyme activity; bile constituents likewise do not inhibit cholinesterase.

The possibility that a disturbance in synthesis of cholinesterase accounted for low serum levels in patients with liver damage was tested by measuring the rate of regeneration of the enzyme in serum following the administration of di-isopropyl fluorophosphate (DFP); this agent has the unique property of inactivating irreversibly the cholinesterases (2). Regeneration rates of serum cholinesterase have already been measured in normal individuals following the administration of this agent (3).

**METHODS.** Studies were carried out on 13 normal subjects and on 15 patients with well-defined liver damage. The latter included 13 patients with cirrhosis, 1 with chronic hepatitis and 1 with carcinoma of the head of the pancreas with biliary obstruction. After the determination of control serum cholinesterase levels of venous blood, 2 mgm. of DFP in peanut oil were injected intramuscularly. This dose was found adequate to destroy virtually all (90-100 per cent) serum cholinesterase activity without significant lowering of red blood cell cholinesterase, and was devoid of undesirable side effects. The extent of cholinesterase inactivation by the drug was determined by the analysis of a blood sample obtained 2 hours after injection. The rate of regeneration was then studied at frequent intervals for a period of at least 2 weeks.

Serum cholinesterase activity was determined by the Warburg manometric technic. The procedure employed was as follows:

The serum was diluted 1:5 with 0.03M sodium bicarbonate; 0.5 cc. of this dilution was placed in the side bulb of the conventional vessel. The main compartment of the flask contained 3.0 cc. of 0.02M acetylcholine bromide and 0.5 cc. of distilled water. The vessels were equilibrated with a mixture of 5 per cent

<sup>1</sup> The work described in this paper was carried out under a contract with the Medical Division of the Chemical Corps U.S.A.

carbon dioxide and 95 per cent nitrogen and allowed to come to thermal equilibrium at 37°C in the water bath. Following an initial reading the vessels were tipped and subsequent readings were made at 10 minute intervals; the results were expressed in absolute values, i.e., cmm. of CO<sub>2</sub> per 0.1 cc. serum per 30 minutes.

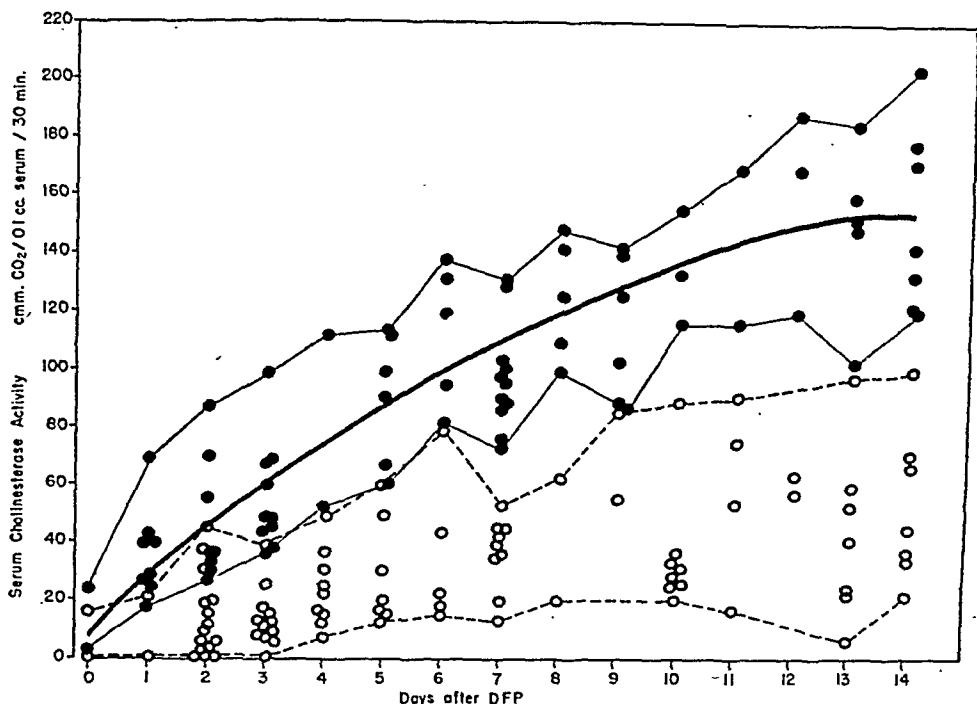


Fig. 1. Regeneration rates of serum cholinesterase in normal individuals and in patients with liver damage.

The solid circles represent values obtained from normal individuals.

The open circles represent values obtained from patients with liver damage.

In both instances the maximal and minimal values of the group are connected to indicate the range of variation for the group.

The heavy solid line represents the mean curve of serum cholinesterase regeneration for the normal group. A mean is not indicated for the patient group.

On days 0 and 1 there were 15 observations on patients with liver damage, and on day 0, 10 observations on normal subjects. For these days only the highest and lowest values are shown.

**RESULTS.** The control serum cholinesterase values of 13 normal individuals ranged from 168 to 242 cmm., with an average value of 202 cmm. The sera of 15 patients with liver damage showed initial levels varying from 18 to 122 cmm., with an average value of 66 cmm. The decreased level of serum cholinesterase in patients with liver disease is in accord with previous findings. (4, 5, 6).

The regeneration rates of the serum cholinesterase of the normal subjects following the administration of DFP are shown in the accompanying figure. The mean curve is parabolic and indicates a more rapid rate of regeneration during the first 7 days. After 14 days the average normal curve reaches 76 per cent of the initial level.

The regeneration rates of the serum cholinesterase after the administration of DFP as determined on the 15 patients with liver damage are shown in the same figure. The rates of regeneration are significantly lower than that of the mean normal. Although during regeneration there is a considerable spread in the absolute values of the serum cholinesterase in both groups, overlap is minimal and is confined to the first 2-3 days of regeneration.

No attempt was made to correlate liver function tests with the rates of regeneration; however the depression in the rate of cholinesterase regeneration appeared to be roughly proportional to the severity of the liver damage.

DISCUSSION. It is known that DFP is rapidly destroyed in vitro and in vivo (7). Therefore the recovery of serum cholinesterase activity is not representative of a reversal of enzyme inhibition but is indicative of synthesis of new enzyme protein. Since the regeneration rate of serum cholinesterase in patients with liver damage is significantly depressed as contrasted to the normal, it is concluded that the ability of such patients to synthesize this particular enzyme protein is decreased. This constitutes further evidence for the view that the liver is a primary locus for the formation of serum cholinesterase.

#### SUMMARY

1. Serum cholinesterase activity in patients with liver disease is depressed below normal levels.
2. Subsequent to the administration of DFP the regeneration rate of serum cholinesterase in patients with liver damage is significantly lower than in normal subjects.
3. These data offer further proof for the postulation that the liver is the primary site of formation for serum cholinesterase.

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# THE INFLUENCE OF ACUTE HYPOTHERMIA ON THE RATE OF OXYGEN CONSUMPTION AND GLYCOGEN CONTENT OF THE LIVER AND ON THE BLOOD GLUCOSE<sup>1</sup>

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A renewal of interest in the physiology of acute hypothermia began with the introduction of general cooling for the treatment of malignancy (1) and schizophrenia (2). The exposure of military personnel to cold during the war served to arouse further interest in hypothermia and the limits within which resuscitation measures are effective. The extensive German investigations on hypothermia have been summarized in the report by Alexander (3). As part of a general study of the physiologic effects of hypothermia in mammals, Crismon (4) reported changes in heart rate, arterial pressure and electrocardiograms of rats during acute reduction of body temperature. In order to obtain information concerning the carbohydrate metabolism in the hypothermic rat we have investigated the changes occurring in liver glycogen, liver oxygen consumption and blood glucose during the period of hypothermia. The effects of glucose,  $\text{Ca}^{++}$  and adrenal cortical hormone administration on the course of hypothermia in the rat are to be presented in a subsequent paper.

**METHODS.** Adult male albino rats of the Slonaker-Wistar strain were anesthetized with 37.5 mgm. per kgm. sodium pentobarbital given intraperitoneally. All experiments were begun in the morning using rats which had been allowed constant access to Purina dog chow and water, or which had been segregated at the appropriate time into cages supplied with water but without food. The anesthetized rats were cooled by placing them on a coil of copper tubing through which water at about 7°C. was circulated. Cooling was continued at the rate of approximately 5°C. per hour until respiration ceased. Details of the cooling procedure have been given by Crismon (4).

In some animals samples of liver tissue were taken before induction of hypothermia and again within five minutes after the last respiratory movement at the termination of cooling. In other animals only terminal liver samples were taken. The procedure for obtaining the initial liver samples was as follows:

A midline incision was made extending about 2 cm. posteriorly from the xiphoid process. The left lateral lobe of the liver was then withdrawn with gentle traction and placed upon a linoleum block inserted under the margin. A wedge-shaped piece of liver, approximately 1 cm. on each side, was removed by means of two strokes of a sharp razor blade. The area from which the sample was taken was painted with the plasma-cell extract mixture described by Sano (5) or fibrin foam saturated with thrombin solution was applied. The cut

<sup>1</sup> Supported by grants from the John and Mary R. Markle Foundation and the Research Fund of the Stanford University School of Medicine.

edges of the liver were held firmly together for 1 to 2 minutes. The skin incision was closed with clips. Examination was always made at the conclusion of the experiment to ascertain whether or not bleeding had occurred. No evidence of hemorrhage was found in any of the animals.

A 100 to 200 mgm. sample of liver was weighed rapidly on a micro-torsion balance and introduced into a centrifuge tube containing hot KOH. Glycogen was determined in this sample by the method of Good, Kramer and Somogyi (6) with the use of  $\text{H}_2\text{SO}_4$  instead of HCl (7). Glycogen is expressed in terms of total reducing substance determined as glucose by the method of Folin (8). The remainder of the liver sample was transferred to a cold moist chamber (9) for the preparation of tissue slices for determination of oxygen consumption. The technique was that previously described (10). The rates of oxygen consumption are expressed as  $\mu\text{l. O}_2$  consumed at  $37.7^\circ\text{C.}$  per mgm. glycogen-free dry tissue. Approximately five minutes elapsed between removal of the liver sample and the beginning of thermo-equilibration of the slices.

Blood glucose was determined by the method of Folin (8) on 0.1 ml. of arterial blood deproteinized with  $\text{H}_2\text{SO}_4\text{-Na}_2\text{WO}_4$ . One-half milliliter of protein-free filtrate was used with one-fourth the usual amount of reagents and the color intensity determined with a Klett-Summerson colorimeter after dilution to 6.25 ml.

**RESULTS AND DISCUSSION.** *Oxygen consumption of liver slices.* The circulatory failure in acute hypothermia, culminating in death, is characterized by a phase of regional asphyxia when the rectal temperature falls below  $19^\circ\text{C.}$  Impaired ventricular contraction, atrio-ventricular block and respiratory failure occurring below this temperature are all signs of such regional asphyxia (4). Hepatic changes following anoxia resulting from hemorrhage (11, 12) or exposure to reduced atmospheric pressure (13) have been shown to result in a decreased  $Q_{O_2}$  of excised liver. It was considered advisable to investigate the oxygen consumption of liver at the conclusion of cooling in order to ascertain whether possible anoxic changes would also result in a decreased  $Q_{O_2}$  of liver from animals during the terminal phase of hypothermia.

For fed animals, in which liver glycogen is markedly influenced by cooling, it is important that comparisons be made using  $Q_{O_2}$  expressed on a glycogen-free basis ( $Q_{O_2}GF$ ) (10).

Table 1 shows the  $Q_{O_2}$  of liver samples removed at the time of the last observed respiratory movements, as well as the temperature at which this occurred, for the following groups of animals:

A. Control animals allowed access to food. Liver samples taken after decapitation without anesthesia or cooling.

B. Control animals fasted 24 hours. Liver samples taken after decapitation without anesthesia or cooling.

C. Cooled animals allowed access to food until the beginning of cooling.

D. Cooled animals fasted 24 hours preceding cooling.

E. Cooled animals given  $\text{CaCl}_2$  intramuscularly during cooling.

F. Cooled animals given 3 ml. 50 per cent glucose by stomach tube during cooling.

G. Cooled animals allowed access to food before cooling and given adrenal cortical hormone. Of the 5 animals one received 1 ml. ACH immediately before cooling, the second received 1 ml. ACH before cooling and 1 ml. on the preceding day, the third received 1 ml. ACH before cooling and 1 ml. on each of the two preceding days, etc.

H. Cooled animals fasted before cooling and given ACH as in group G. The fasting periods were 24, 48, 72, 96 and 120 hours.

I. Cooled animals adrenalectomized one week before cooling and maintained on NaCl.

TABLE 1

*Oxygen consumption of rat liver slices from control animals and from animals at the termination of cooling with and without various forms of treatment*

GROUP	NUMBER OF ANIMALS	MEAN TERMINAL TEMP.*	MEAN $Q_{O_2}$ GF†	S.E.	P‡
Uncooled controls					
A Fed	41	°C.	9.11	0.14	
B Fasted 24 hrs.	6		8.82	0.20	<0.1
Cooled animals					
C Fed	10	14.4	8.69	0.22	0.2
D Fasted 24 hrs.	6	9.6	9.84		<0.7
E $CaCl_2$ intramusc.	3	9.8	9.11		0.9
F Glucose	5	9.7	9.44	0.16	0.3
G Fed + ACH	5	10.4	9.84	0.26	<0.05
H Fasted + ACH	5	13.4	10.29	0.30	<0.001
I Adrenalectomized	5	16.7	8.86	0.21	0.4
J Adrenalect. + ACH	4	11.9	8.48	0.06	<0.05

\* Mean rectal temperature at which respiration ceased.

† Mean  $Q_{O_2}$  expressed on a glycogen-free basis.

‡ Probability that the mean in question could have come from the population from which the mean of group A (uncooled, fed controls) was obtained.

J. Cooled animals adrenalectomized one week before cooling, maintained on NaCl, and given 2 ml. ACH before cooling.

The animals in groups E to J are included in this series so that the oxygen consumption of liver slices from them may be compared with control animals. The details of the procedures used and the effect of these procedures on the course of hypothermia will be considered in a subsequent paper.

In order to assess the influence of cooling upon the oxygen uptake of the liver the  $Q_{O_2}$  of the liver from animals of group A (uncooled, fed controls) was compared with the  $Q_{O_2}$  of the liver from animals of other groups using the "t" test of Fisher (14). The values for P listed in table 1 indicate the probability that the difference in  $Q_{O_2}$  between a given group and group A would occur through errors in random sampling. It is clear from the table that in no instance was

the  $Q_{O_2}$  of the liver significantly impaired by hypothermia. In some cases, particularly those groups receiving ACH, the  $Q_{O_2}$  was increased above that of the controls.

TABLE 2

*Rate of oxygen consumption and glycogen content of the liver and blood glucose of rats before and at termination of acute hypothermia*

ANIMAL NO.	RECTAL TEMP.	TIME	LIVER WATER	LIVER GLYCOGEN	LIVER $Q_{O_2}$	LIVER $Q_{O_2}GF$	BLOOD GLUCOSE
Fed animals							
1	°C.	min.*	%	%			mgm. %
	36.5	23		5.04	8.15	9.76	158
	14.5	305	71.6	0.33	8.80	8.89	138
2	36.5	20		1.79	7.50	7.98	144
	15.4	225	72.4	0.22	9.74	9.78	88
3	35.5	20		4.08	6.98	8.04	114
	14.0	355	70.5	1.45	8.76	9.20	200
4	36.0	40		3.93	6.70	7.68	146
	13.5	285	69.8	0.37	6.80	6.88	160
5	35.0	96		3.92	8.22	9.41	126
	14.0	217	71.6	1.49	8.77	9.23	250
Animals fasted 24 hours							
6	33.0	42		0.05	5.10	5.11	70
	9.5	492	71.3	0.01	9.21	9.21	61
7	38.0	42		0.17	7.13	7.17	97
	10.0	374	71.6	0.03	9.35	9.35	52
10	37.7	16		0.04	9.65	9.66	117
	9.4	342	70.2	0.02	10.62	10.62	68
Animal fasted 48 hours							
9	37.5	65		0.36	7.58	7.67	100
	12.4	414	69.1	0.03	8.11	8.11	85

\* Time in minutes from administration of anesthetic.

$Q_{O_2}$  =  $Q_{O_2}$  on dry weight basis without correction for glycogen.

$Q_{O_2}GF$  =  $Q_{O_2}$  of a glycogen-free dry weight basis (10).

The oxygen consumption of liver slices removed before cooling and at the termination of cooling of the same animal is given in table 2. The mean initial  $Q_{O_2}GF$  for the liver of the fed animals is 8.55 and the final 8.77. This difference is not significant ( $P = 0.9$ ). If the fasted animals are considered as a group the mean initial  $Q_{O_2}GF$  is 7.40 and the final 9.32. This difference is statistically

significant ( $P = 0.02$ ). In table 1 the  $Q_{O_2}GF$  of liver from fasted, uncooled rats was 8.82, a value considerably higher than that found when the liver sample was obtained by biopsy. It appears probable that excessive trauma, resulting from slicing the very small initial sample, resulted in sufficient cell damage to reduce the  $Q_{O_2}$  of the initial samples in both the fed and fasted animals given in table 2.

The data given in tables 1 and 2 indicate no tendency toward a decrease in liver oxygen consumption following cooling as might be expected to result if an important degree of asphyxia occurred during hypothermia. During the course of this study 5 animals were observed in which asphyxia probably occurred. The  $Q_{O_2}$  of liver samples from these animals is given in table 3. In all instances the  $Q_{O_2}$  was markedly lower than that from cooled animals in which the course

TABLE 3

*Oxygen consumption and glycogen content of liver from hypothermic rats in which asphyxia probably occurred*

ANIMAL NUMBER	FINAL RECTAL TEMP.	FINAL LIVER GLYCOGEN	FINAL LIVER $Q_{O_2}GF$	REMARKS
	°C.	%		
A-3S	24.1	0.16	3.02	Inadequate artificial respiration 134 min. during rewarming from 16°C.
A-42	20.3	1.09	6.53	Ether. Rapidly cooled
A-43	25.0	0.06	6.19	Ether. Rapidly cooled
A-45	14.5	0.25	5.34	Lanatoside-C. Sample from recently infarcted area
A-51	14.6	2.28	6.22	Lanatoside-C. Sampled 26 min. after cessation of respiration

of hypothermia was uneventful (table 1, group C). It is thus clear that reduction in  $Q_{O_2}$  of liver may occur during asphyxia in hypothermic animals as well as in animals at normal body temperature (11, 12, 13).

It seems possible that striking reduction in rate of local gas exchanges may occur in hypothermic animals although the demonstration of consequences of hepatic asphyxia under these conditions is lacking. Since details of liver metabolism during hypothermia and during the subsequent period of measurement of  $Q_{O_2}$  at 37.7°C. are not known, the observation of normal to high  $Q_{O_2}$  permits no definite conclusions regarding rates of gas exchange in the cold. In view of the decrease in rate of oxygen consumption of liver at low temperatures *in vitro* (10) a moderate decrease in absolute rate of gas exchange in the hypothermic animals would not be expected to produce functional changes. Functional changes, as indicated by decreased  $Q_{O_2}$  of excised liver, would be expected to occur only under conditions in which the metabolic demands of the tissue

exceeded the supply of oxygen. If asphyxia be defined in terms of gas exchange, no conclusion may be drawn; if, however, damage from decreased rate of gas exchange be taken as the criterion, then it must be concluded that asphyxia did not occur. In this distinction lies a metabolic demonstration of a protective action of cold.

*Blood glucose and liver glycogen.* The blood glucose concentration was determined in samples of tail blood taken from animals before induction of anesthesia and in 4 to 6 additional samples of carotid artery blood from each animal during the course of cooling. In table 2 are given only the pre-cooling and terminal blood glucose concentrations. The intervening concentrations of blood glucose indicated that the final level was attained as the result of a progressive rise or fall from the initial level: marked fluctuations in blood glucose did not occur.

In table 2 are also given the initial and final concentrations of liver glycogen for the animals in which blood glucose was determined. Further information concerning the effect of hypothermia on the liver glycogen concentration may be derived from a comparison of the liver glycogen in fed and fasted uncooled

TABLE 4  
*Effect of hypothermia on liver glycogen concentration*

GROUP	TREATMENT	NUMBER ANIMALS	LIVER GLYCOGEN	S.E. MEAN
			%	
A	Uncooled, fed	24	4.10	0.236
C	Cooled, fed	14	0.83	0.187
B	Uncooled, fasted 24 hrs.	6	0.08	
D	Cooled, fasted 24 hrs.	4	0.02	

animals with that in cooled animals of similar nutritional status (table 4). In the fed animals, in which the liver glycogen level is initially high, reduction of body temperature to 14–16°C. (the point of respiratory arrest) results in the loss of approximately 80 per cent of the liver glycogen, or about 3.7 grams glycogen per 100 grams of liver. In animals with liver glycogen greatly depleted by fasting, cooling until cessation of respiration (9–10°C.) results in about the same percentage loss of glycogen (75 per cent), but the absolute loss is only about 0.06 gram per 100 grams of liver.

It has been known for many years that a rapid depletion of liver glycogen occurs during hypothermia (15, 16, 17, 18, 19). The data reported here become more informative in regard to carbohydrate metabolism during hypothermia if they are considered together with coincidental changes in blood sugar. It may be seen from table 2 that in the fasted animals and in 3 of the 5 fed animals the blood glucose either declined during cooling or remained relatively constant. Two of the fed animals, however, showed marked rises in blood glucose during cooling. In both of these animals (nos. 3 and 5, table 2) the liver contained considerable glycogen at the time of death. The periods of rapid rise in blood glucose concentration in animals 3 and 5 coincided in time with the periods of

most rapid reduction in body temperature. Rat 3 was cooled at the rate of  $0.196^{\circ}\text{C.}$  per minute for 35 minutes, during which the blood glucose increased from 112 to 195 mgm. per cent. Rat 5 was cooled at the rate of  $0.208^{\circ}\text{C.}$  per minute for 94 minutes, during which the blood glucose increased from 126 to 189 mgm. per cent. These rapid rates of cooling were not attained in the other animals studied. The mechanisms governing the direction of change in blood glucose during hypothermia are discussed below.

DISCUSSION. In 1855 Claude Bernard (15) reported hyperglycemia and glycosuria in animals cooled to a body temperature of about  $20^{\circ}\text{C.}$  by immersion in ice water. Since that time others have also reported that hyperglycemia occurs during hypothermia (17, 18, 20, 21, 22). Recently Alexander (3) commented upon the finding of hyperglycemia in one patient rescued from the sea during the war. Data presented by Holzlöhner, Rascher and Finke (23) on human subjects at Dachau show that the blood sugar rises as body temperature is reduced. In five experiments the mean blood glucose rose from 87 to 178 mgm. per cent during reduction in body temperature from  $37^{\circ}$  to  $26.6^{\circ}\text{C.}$ , and then fell again during rewarming.

Hypoglycemia has been reported by some workers in experiments in which the animals were cooled slowly and for prolonged periods (22, 16, 18). Smith and Fay (1) reported low blood sugar in man during prolonged general cooling. Silvette and Britton (24) found hypoglycemia to occur in cats wetted and exposed to cold. More recently Hartman and Brownell (25) studied the response of adrenalectomized and normal cats to chilling and found essentially the same responses of blood sugar in both groups. There was usually a preliminary rise, followed by a fall to levels which were above those producing hypoglycemic convulsions.

The rapid, and in some cases practically complete, utilization of liver glycogen which was observed following acute hypothermia was to be expected. The method used for obtaining liver samples, however, made it possible to derive this information from initial and terminal determinations in one animal rather than relying entirely upon inter-animal comparisons. The use of barbiturate anesthesia has been reported to depress glycogen formation in the liver (26). In the experiments reported here this difficulty has been circumvented partially by allowing the animals to recover from the anesthetic to a considerable degree before beginning cooling (4).

During the initial phase of hypothermia oxygen consumption of the intact animal is greatly increased (22, 27). In man metabolic rates as high as 3.59 times the BMR have been reported during general hypothermia (19). The high metabolic rate is a result of intense shivering and increased muscle tonus during this period. Blood lactic acid is increased (22, 17) and muscle glycogen is depleted (18). During this period rapid glycogenolysis must occur also in the liver. The blood sugar level under such conditions is chiefly the resultant of utilization and glycogenolysis; the rôle of glyconeogenesis during hypothermia is uncertain. Provided the animal's stores of carbohydrate are sufficient, hyper-

glycemia may occur. With further reduction in body temperature shivering ceases, skeletal muscle tonus is reduced and the rate of glucose utilization is greatly diminished. Hyperglycemia occurring during the early stages of hypothermia is thus maintained during the later stages. If the initial level of liver glycogen is low, as in fasted animals, hyperglycemia fails to occur during the early stages of hypothermia, and progressive fall in blood sugar is found throughout the period of cooling.

The most striking observation made during this investigation was the improved survival in hypothermia of fasted animals. Rats starved for 24 hours continued to breathe spontaneously until the rectal temperature reached  $5.8^{\circ}$  to  $9.8^{\circ}\text{C}.$ , while those which were fed stopped breathing at  $13.3^{\circ}$  to  $16.4^{\circ}\text{C}.$  This influence of fasting on the course of hypothermia has been studied more completely and is to be reported in a separate communication.

#### SUMMARY

1. Adult albino rats, lightly anesthetized with sodium pentobarbital, were cooled until respiration ceased. Liver samples for the determination of glycogen content and oxygen consumption were taken before cooling and again at termination. Blood glucose was determined during the course of cooling.

2. The oxygen consumption of liver, expressed on a glycogen-free basis, was not significantly decreased as the result of cooling or of asphyxia except in 5 animals in which asphyxia was considered probably to have occurred.

3. Liver glycogen was rapidly utilized during the period of hypothermia. In animals provided with ample stores of carbohydrate at the initiation of cooling, blood glucose increased during the early phases of hypothermia and remained elevated. In fasted animals and in slowly-cooled fed animals the blood glucose was maintained or fell during the course of cooling.

4. Animals fasted 24 hours could be cooled to a mean rectal temperature of  $9.6^{\circ}\text{C}.$  before respiration ceased, while those permitted previous access to food could be cooled only to  $14.3^{\circ}\text{C}.$  before respiratory arrest occurred.

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# PLASMA AND LIVER PROTEIN CONCENTRATIONS OF RATS FED THIOURACIL<sup>1</sup>

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A serum or plasma globulin concentration above the normal level is invariably observed in rats in hypothyroidism induced by hypophysectomy, thyroidectomy, and after the feeding of antithyroid drugs (1-4). Electrophoretic analysis of the serum substantiated the results obtained by the salt fractionation methods (4-6) and revealed that the  $\alpha$  globulin component is present after removal of the thyroid or hypophysis but is rarely present in normal rat serum (4-5). Plasma albumin changes in the hypothyroid state, however, appear to be a reflection, primarily, of the nutritional state of the animal. The subnormal plasma albumin concentrations observed in rats fed thiourea (0.5 per cent) were found to be due to reduced food intake (7) and this may be the cause, in part at least, for the low albumin levels in hypothyroid patients (8). Recently, Moore, Levin and Smelser (4) reported a significant rise in serum albumin after thiouracil feeding, a finding we have not observed with thiourea. Therefore, our current study is, in part, an investigation of the plasma protein levels in rats fed thiouracil.

Since the liver is considered to be a source of plasma albumin, experimental conditions that influence plasma protein concentrations might also influence liver protein concentrations. In this regard, it was of interest first of all to re-examine liver weights from rats fed antithyroid drugs since a reduction in food intake will tend to decrease liver weight due, in part at least, to a loss in protein (9-10). Despite the reduction in food intake caused by the inclusion of thiourea in the diet, liver weight was found to be greater in the hypothyroid rats although the significance was border line (3, 11). On the other hand, May, Moseley and Forbes (12) did find a significant increase in liver weight in rats fed thiourea for a more extended time period. Leblond and Hoff (13) also have commented on the fact that liver atrophy due to thyroidectomy is not observed in a thiouracil induced hypothyroid state. In this report it is shown that a relative increase in liver weight and protein content over that of pair fed control rats does occur in rats fed thiouracil for 20-25 days.

**MATERIALS AND METHODS.** Male rats of the Long-Evans strain, ranging from 135-150 days in age were used. The animals were kept in pairs in metabolism cages for measurement of daily food intake. The stock diet of Purina calf meal supplemented with 10 per cent meat scrap (containing 55 per cent protein) was available for ad libitum feeding when the diet contained thiouracil.<sup>2</sup> The drug

<sup>1</sup> This investigation was done under contract with the Office of Naval Research, Navy Department.

<sup>2</sup> Thiouracil (Deravet) was generously supplied by Dr. Mark Welsh of Lederle Laboratories, Pearl River, New York.

was fed as one-half of one per cent of the diet and since this caused a voluntary reduction in food intake the control animals on stock diet were pair fed. The diet was supplemented by a mixture of  $\frac{1}{3}$  Mazola and  $\frac{2}{3}$  cod liver oil on bread twice weekly and by fresh carrots once each week.

Thiouracil was fed for periods of 20-25 days at which time the rats were lightly anesthetized with ether and bled from the heart. The methods used for determining hematocrit, non-protein nitrogen, total plasma protein, albumin and globulin were the same as those reported previously (3). Each rat was autopsied and the fresh weight of the pituitary, adrenals, thyroids, kidneys and liver was recorded. The liver was dried to constant weight at 95°C to determine water content and was then ground to uniform consistency and analyzed for total nitrogen. Nitrogen values were converted to protein by use of the factor 6.25.

RESULTS AND DISCUSSION. The addition of thiouracil to the diet had a tendency to reduce food intake rather markedly for the first few days only. Total

TABLE 1  
*Influence of thiouracil (0.5%) on body weight and plasma protein levels of rats*

NO. OF RATS	TREATMENT	BODY WEIGHT START-END	HEMATOCRIT	NON-PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN
		gram	per cent	mgm/100 cc.	gram/100 cc.	gram./100 cc.	gram/100 cc.
14	thiouracil	336-326	44.4 ± 0.5*	66.0 ± 1.7	6.81 ± 0.09	3.26 ± 0.08	3.55 ± 0.17
14	normal (pair fed)	336-315	48.7 ± 0.6	56.0 ± 1.1	6.04 ± 0.11	3.19 ± 0.08	2.85 ± 0.14

$$* \epsilon = \sqrt{\frac{\sum d^2}{N(N-1)}}$$

food consumption per rat during the first 20 days ranged between 271 and 355 grams although only two rats ate more than 300 grams. The food consumption represents a subnormal amount when compared with ad libitum fed rats as they eat 330 grams or more. The reduction in food intake was indicated by the average 10 gram loss in body weight (table 1). Since pair fed rats lost an average 21 grams during the same period it would appear that food intake is the causative factor for body weight loss. The effect of thiouracil (0.5 per cent) on body weight is much less severe than that observed when thiourea is fed at the same level and differs from thiourea which seemed to accentuate the loss in body weight beyond that caused by a voluntary reduction in food intake (11).

Thiouracil (0.5 per cent) induced changes in the plasma protein concentrations which simulated the results obtained by feeding thiourea at the same level. Total plasma protein, plasma globulin concentrations and non-protein nitrogen were elevated significantly by thiouracil whereas the plasma albumin concentration was unaltered in comparison with pair fed controls (table 1).

It should be noted that a decrease in hematocrit was recorded and if a correction for this decrease was permissible then the plasma albumin levels would be significantly greater than in pair fed controls. Actually, the plasma albumin concentrations were somewhat below those obtained when rats are fed the stock

diet ad libitum (3) and a correction for hematocrit decrease would not fully compensate for the change. Therefore, we have not observed the above normal albumin levels (concomitant with increased levels of globulin) recorded by Moore, Levin and Smelser (4) with 0.2 per cent thiouracil feeding.

With the exception of the anticipated increase in thyroid weight, thiouracil did not significantly influence the weight of the pituitary, adrenals or kidneys as compared with pair fed controls when the data are considered either as actual weight or as grams/100 grams body weight (table 2). Similar results were obtained with thiourea (3). Liver weight, however, was significantly greater in thiouracil fed rats (table 2). The increase in liver weight has been noted previously by Leblond and Hoff (13) by using thiouracil and by May, Moseley and Forbes (12) by using thiourea.

TABLE 2  
*Influence of thiouracil on organ weights of rats*

TREATMENT (NO. OF RATS)	AVE. ORGAN WEIGHT MGM.		AVE. ORGAN WEIGHT MGM. PER 100 GRAM BODY WEIGHT	
	Normal (14)	Thiouracil (14)	Normal (14)	Thiouracil (14)
Pituitary.....	9.6 $\pm$ 0.9	11.3 $\pm$ 1.1*	2.9 $\pm$ 0.2	3.4 $\pm$ 0.3
Adrenal.....	31.7 $\pm$ 2.1	27.5 $\pm$ 2.5	9.9 $\pm$ 0.8	8.1 $\pm$ 0.8
Thyroid.....	23.6 $\pm$ 2.2	44.7 $\pm$ 2.1	7.4 $\pm$ 0.8	12.7 $\pm$ 0.7
Kidney.....	2990.0 $\pm$ 191	2607.0 $\pm$ 182	936.0 $\pm$ 71	803.0 $\pm$ 69
Liver.....	10749.0 $\pm$ 304	13545.0 $\pm$ 389	3347.0 $\pm$ 95	4155.0 $\pm$ 118

$$* \epsilon = \sqrt{\frac{\Sigma d^2}{N(N-1)}}$$

Since total plasma protein is increased by antithyroid drugs, it was deemed of interest to determine whether liver protein was influenced by thiouracil. A series of adult male rats were fed 0.5 per cent thiouracil for 20 days by including the drug in the diet and plasma and liver proteins were compared with pair fed controls. The effect of the antithyroid drug on body weight, hematocrit, NPN, and the plasma protein concentrations was a virtual duplication of our first series (tables 1 and 3). Liver weight was increased, being  $15189 \pm 976$  mgm. after thiouracil and  $10904 \pm 576$  mgm. in the controls. Approximately 70 per cent of the liver weight in both groups was due to water (table 3) so it is apparent that liver weight differences were not caused by this organ component. It was found, however, that liver protein in grams/100 grams body weight was significantly more abundant in rats fed thiouracil than in their respective controls (table 3). The actual percentage of protein comprising the dry weight of the liver was not changed significantly, being  $66.9 \pm 1.2$  per cent in thiouracil fed rats and  $69.9 \pm 1.7$  per cent in control rats. Therefore, despite a decrease in body weight due no doubt to a reduced food intake, liver weight was maintained or increased and the percentage protein did not change resulting in an increased amount of liver protein in the body per unit of weight.

May and co-workers (12) found liver glycogen markedly increased after thiourea administration but neutral fat and cholesterol were unchanged. They feel

that the increase in liver glycogen may contribute to the liver weight increase and our data show that it is not water accumulation or a protein loss.

The profound effect of the antithyroid drugs on the liver warrants the mention of other investigations in which a relationship between thiouracil and the liver seems apparent. For example, György and Goldblatt (14) found that thiouracil has a preventative action against the production of cirrhosis of the liver by dietary means and recently, Cantarow et al. (15) reported that induction of hepatic lesions by 2-acetaminofluorene was markedly influenced by thiouracil in that the antithyroid drug exerted a protective action. Further investigation is necessary to provide a more fertile field for speculation.

TABLE 3  
*Plasma and liver protein concentrations in rats fed thiouracil*

RATS	TREATMENT	BODY WEIGHT CHANGE	PLASMA PROTEINS			LIVER	
			Total protein	Albumin	Globulin	Water	Protein
			grams/100 cc.	grams/100 cc.	grams/100 cc.	per cent	grams/100 grams B.W.
7	thiouracil	-8	6.68 ± 0.12*	3.39 ± 0.15	3.29 ± 0.16	71.0 ± 0.3	0.888 (0.806-1.053)
7	normal (pair fed)	-18	5.75 ± 0.08	3.11 ± 0.14	2.63 ± 0.19	70.1 ± 0.6	0.719 (0.652-0.787)

$$* \epsilon = \sqrt{\frac{\Sigma d^2}{N(N-1)}}$$

#### SUMMARY

A rise in plasma globulin concentration, in total plasma protein and in non-protein nitrogen occurred in male rats fed a half of one per cent thiouracil for 20 to 25 days. Plasma albumin concentrations simulated those of pair fed controls. The hematocrit was decreased.

Despite a slight decrease in body weight resulting from a voluntary reduction in food intake, the livers from thiouracil fed rats were heavier than those from controls. Since the percentage water and protein remained unchanged, this resulted in an increase in liver protein in the body per unit of weight.

The weight of the pituitary, adrenals and kidneys was not significantly altered.

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# RESPIRATORY AND CIRCULATORY CHANGES DURING ACCLIMATIZATION TO HIGH ALTITUDE<sup>1</sup>

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The studies of decreased barometric pressure made by Paul Bert (1) during the latter half of the nineteenth century may properly be considered as the beginning of modern knowledge of, and interest in, the effects of oxygen lack upon the human body. During the same period the rapid development of mountaineering as a sport produced a new occupational disease—altitude sickness, or *seroche* as it was called in the Andes. At the turn of the last century Mosso (2) was defending acapnia as the cause of mountain sickness, against Haldane and Priestley (3), who regarded acapnia as the result rather than the cause of the illness. About this time it was observed that residents at high altitude experienced none of the unpleasant symptoms developed by visitors recently arrived, and a process of acclimatization to high altitude was postulated.

In the famous controversy which raged for more than a quarter of a century, stimulating two scientific mountain expeditions and innumerable laboratory studies, Haldane and Priestley (3) contended that acclimatization was due, in part at least, to active oxygen secretion from alveolus to blood, whereas Barcroft (4) maintained that oxygen secretion never occurred, and argued that acclimatization consisted of a series of respiratory and circulatory changes. Our modern knowledge indicates that Barcroft's concept, fully discussed by him in 1934 (5) and recently summarized by Van Liere (6) is correct. The Pike's Peak expedition of 1913 (7) which remained for five weeks at 14,100 feet, demonstrated a fall in the "alkaline reserve" of the blood, as previously suspected by Galleotti (8), and confirmed the earlier observations by Viault (9) of the increase in circulating red blood cells. The Pike's Peak investigators mistakenly believed that they had proven the occurrence of oxygen secretion by the lung. A thorough analysis of the process of acclimatization was made by the 1922 expedition to Peru (10). Barcroft, the leader of this expedition, considered the three major factors to be: increased pulmonary ventilation, polycythemia and a shift to the left of the oxyhemoglobin dissociation curve. The International High Altitude Expedition of 1936 greatly expanded the original studies and added detailed analyses of electrolyte balance, blood pH, and the characteristics of hemoglobin (11, 12). Most of these mountain studies were carried out between 13,000 and 18,000 feet.

In the meantime mountaineers, climbing solely for sport and unable to perform

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complex measurements, ascended to 28,000 feet on Mt. Everest in 1924 (14) and on numerous occasions spent weeks above 22,000 feet (13, 15). Their contribution to our knowledge of acclimatization may be summarized as follows: a gradual ascent is necessary to provide complete acclimatization; secondly, at extreme altitudes, deterioration may be as rapid as is acclimatization (although cold, fatigue, and inadequate food and shelter influence this effect); thirdly, acclimatized man can perform heavy work as high as 25,000 feet, an altitude which rapidly causes unconsciousness in unacclimatized man; and finally, additional oxygen does not fully restore the acclimatized man to his sea level condition (14).

All of these high altitude expeditions had one common defect: the subjects were also the observers, and since oxygen lack notoriously dulls cerebral function, their observations of their own and their companions' condition were open to question. Ideally, only the subjects should be exposed to oxygen lack, while the observers should be unaffected, a condition attainable only by use of a low pressure altitude chamber. Brief studies of this type were made by Hasselbalch and Lindbard (16), Haldane et al. (17) and Barcroft (5), but their work was necessarily limited in time and scope. Only the extensive development of altitude chambers and the availability of trained personnel attendant upon the altitude training programs of the last war, made possible the prolonged study of acclimatization which is reported here.

In the course of this study a wide variety of data were collected. The present report, however, deals only with those data required for an analysis of the oxygen transport system and some closely related adaptations.

**EXPERIMENTAL DESIGN.** From a number of volunteers were selected four subjects found to be physically sound and psychologically stable.

*McNutt:* Age 27, height 175 cm., weight 69 kgm., surface area 1.82 sq. m. College graduate (physiology), excellent and well trained athlete, non-smoker.

*Morris:* Age 19, height 180 cm., weight 69 kgm., surface area 1.86 sq. m. High school graduate, moderate athlete, smokes one package cigarettes daily.

*Hertel:* Age 23, height 174 cm., weight 66 kgm., surface area 1.79 sq. m. High school graduate, moderate athlete, smokes one package cigarettes daily.

*Wilkins:* Age 20, height 171 cm., weight 62 kgm., surface area 1.72 sq. m. High school graduate, moderate athlete, smokes one package cigarettes daily.

A rectangular altitude chamber measuring 10 by 12 by 7 feet was fitted with conveniences necessary to make living conditions as comfortable as possible. By means of a communicating lock, in which pressure could be equalized with either the main chamber or the outside, observers wearing oxygen equipment could enter the chamber at will without themselves experiencing anoxia. The pressure within the chamber was accurately controlled by manually operated valves, adjustment of which permitted continuous ventilation of the chamber at a rate of 20-30 cubic feet per minute. A Pauling (18) oxygen analyzer, installed within the chamber, was read several times each day and checked at least once daily by analysis of a sample of the chamber air by the Haldane technic. The pressure was carefully determined by a mercury barometer which agreed almost precisely with two previously calibrated aircraft altimeters, and at no time did

the analysis of chamber air show significant accumulation either of additional oxygen or of carbon dioxide.

After an initial three day observation period at sea level, the chamber pressure was reduced to simulate an ascent of 2000 feet per day up to 8000 feet, of 1000 feet per day to 15,000 feet, and of 500 feet per day thereafter (fig. 1). Up to 15,000 feet the daily ascents were made in the course of a few minutes; above this altitude the pressure was reduced over a two hour period during the evening. The subjects were usually told of the altitude, and all plans and results were freely discussed with them. Temperature was regulated between 65° and 75°F

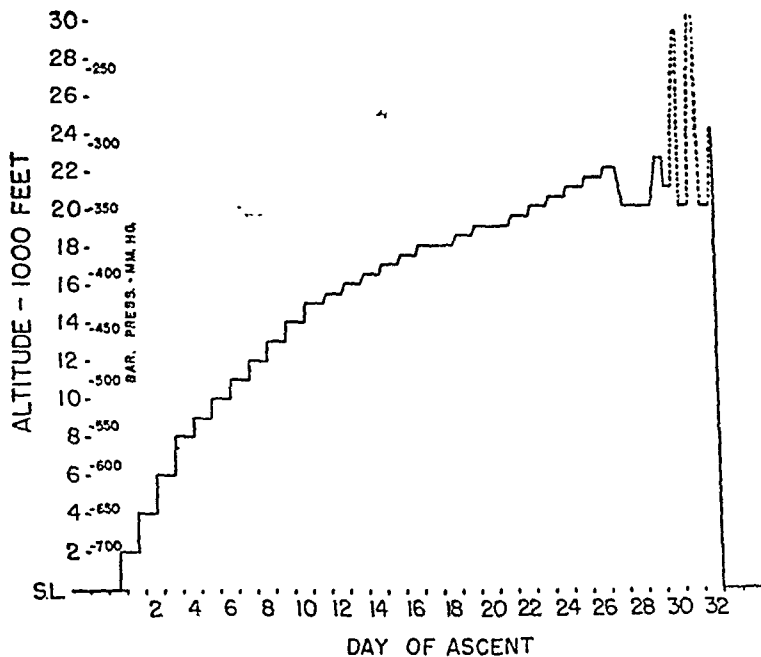


Fig. 1. Rate of ascent.

An observer was continuously on duty at a window to keep detailed notes of the subjects' condition. A complete kitchen under the direction of a trained dietitian was established to provide the subjects with the best available food, and they were permitted to choose their menus. A bicycle ergometer was available within the chamber and McNutt exercised regularly; the others worked only sporadically.

Every effort was made to provide the subjects with comfortable living conditions, appetizing meals, and moderate exercise, in a controlled environment of which the chief variable was the barometric pressure. They were obviously far better provided for than are mountaineers at comparable altitudes. In addition all of the studies and observations were made by trained observers who were themselves unaffected by anoxia. This experimental design is obviously unattainable on mountain expeditions.

Each morning before the subjects arose an observer measured each man's resting pulse and blood pressure and collected a sample of alveolar air by the

Haldane-Priestley method; each man was then weighed. At eight-thirty, approximately one hour after breakfast, the resting arterial blood and respiratory studies described below were begun, followed shortly afterwards by the same studies during standard work. These studies were made on one subject each day. Either x-rays, or psychological tests were then made on all subjects, and before the noon meal an hour was made available for exercise and recreation. A short rest followed the meal, and the remainder of the afternoon was devoted to electrocardiographic work and measurement of pulse rate before, during, and after standard exercise on a twenty-inch step. Baths were given before supper which was usually followed by movies and a visit by a medical officer. Hand-work, cards, books, and a radio supplemented their recreational facilities, and thanks to the whole-hearted cooperation of the entire team, the four volunteers remained in good health and spirits throughout the thirty-five day study.

**METHODS.** The analysis of oxygen transfer from inspired air to tissue capillaries was based upon samples of arterial blood and expired air which were collected in the following manner. An indwelling needle was inserted into the brachial artery under local anesthesia, and a mouthpiece and nose clip were adjusted. After the subject had rested quietly for 10 or 15 minutes, samples of blood and expired air were collected simultaneously during a one minute period. Then, with the arterial needle still in place, the subject mounted a stationary bicycle and pedalled at 69 revolutions per minute in time to a metronome. At sea level the work load was not measured, but at altitude the load was so adjusted that the work was 2530 foot lbs. per minute except at altitudes above 20,000 feet where the work was reduced to 1490 foot lbs. per minute. Samples of arterial blood and expired air were again collected after the subject had been exercising for 7 minutes. In general, each man served as subject every fifth day and the two blood samples totaled 35 ml. The studies caused the men only slight discomfort, and after their first one or two experiences as subject, they were not apprehensive about the procedures.

Pulmonary ventilation, i.e., the minute volume of expired air at body temperature and pressure and saturated with water vapor (BTPS), was calculated from the inspiratory minute volume as measured in a Tissot spirometer. This value, corrected to dry volume at standard sea level temperature and pressure (STPD), was used in the calculation of carbon dioxide output and oxygen intake. The expired air was collected at altitude in a Douglas bag and brought to sea level for analysis for  $\text{CO}_2$  and  $\text{O}_2$  in the Haldane apparatus.

Arterial blood samples were drawn into iced syringes in which the dead space had been eliminated with heparin-fluoride solution. Carbon dioxide and oxygen pressures of the arterial blood were determined by the direct bubble method of Riley et al. (19).

Effective alveolar gas pressures were calculated by the method of Riley et al. (20), in which the effective *alveolar*  $\text{pCO}_2$  is considered to equal *arterial*  $\text{pCO}_2$ , and effective alveolar  $\text{pO}_2 =$

$$\text{tracheal } \text{pO}_2 \times \frac{\text{expired } \% \text{N}_2}{\text{inspired } \% \text{N}_2} - \frac{\text{arterial } \text{pCO}_2}{\text{R.Q.}}$$

To determine the oxyhemoglobin capacity 2 ml. of blood were exposed to 8 ml. of 85 per cent carbon monoxide in a 10 ml. syringe within 7 to 10 minutes after the blood was drawn. The syringe was then rotated for 3 minutes, the CO expelled, fresh CO introduced, and rotation continued for 3 minutes more. The gas was then completely expelled and the syringe sealed. This sample was then analyzed by the Scholander-Roughton technic (21) to obtain the CO capacity of the blood. The O<sub>2</sub> and CO contents of the blood as drawn were determined by the combined method of Scholander and Roughton (21). The O<sub>2</sub> capacity was then calculated from the CO capacity by subtracting the CO content (22).

When blood contains both O<sub>2</sub> and CO it is necessary to take both gases into consideration if pressure-saturation points are to be compared to standard oxyhemoglobin dissociation curves (22). Accordingly, total saturation

$$\frac{(\text{COHb}) + (\text{O}_2\text{Hb})}{(\text{Hb}) + (\text{COHb}) + (\text{O}_2\text{Hb})} \times 100 \quad \text{and}$$

total gas pressure ( $p\text{O}_2 + M_p\text{CO}^4$ ), were calculated for each arterial blood sample. In these experiments, however, the CO content of the blood was uniformly so low that total saturation was but a fraction of 1 per cent higher than oxyhemoglobin saturation and total gas pressure was usually but 1 mm. Hg higher than O<sub>2</sub> pressure. Since these differences are of little significance the oxygen data alone will be used in the graphs and discussions appearing in this paper.

The carbon dioxide content of arterial blood was determined on 0.5 ml. samples in the Van Slyke apparatus. The CO<sub>2</sub> content of fully oxygenated blood at a  $p\text{CO}_2$  of 40 mm. Hg (T40), the CO<sub>2</sub> content of the serum, and the pH of the serum were calculated by the graphic methods contained in the Syllabus of Methods of the Fatigue Laboratory (23). For comparison the pH<sub>s</sub> was also determined with the glass electrode (22).

On two occasions cardiac output determinations were made by the dye injection method of Hamilton et al. (24), with modifications in the sampling technic.

The hematocrit was determined using Wintrobe tubes. Readings were taken after centrifuging the blood for 25 minutes at approximately 3000 r.p.m. Blood sugar was determined by the modified Folin-Malmros method (23). Plasma proteins were determined by the Kjeldahl method (25) and by the copper sulfate specific gravity method (23). The former values averaged about 0.4 gram per 100 ml. higher than the latter and are considered the more accurate. The plasma was also analyzed for non-protein nitrogen, lactic acid, and chloride (23). All analyses on plasma were very generously performed for us by Mr. Frank Consolazio at the Harvard Fatigue Laboratory.

RESULTS. The data are presented in tables 1-5 and in figure 2.

*Pulmonary ventilation* (table 1). In all four subjects the ventilation increased as the altitude increased, though there were marked individual variations. McNutt and Wilkins, for example, showed relatively little increase in ventilation, whereas Hertel and, to a lesser extent, Morris, showed a considerable increase.

<sup>4</sup>M is the relative affinity of CO for hemoglobin as compared to the affinity of O<sub>2</sub> for hemoglobin. Its value is approximately 210.

The effectiveness of a large volume of pulmonary ventilation in sustaining arterial oxygenation during exposure to reduced oxygen pressures (26) is dramatically

TABLE 1

DATE	PRES- SURE ALTI- TUD (THOU- SANDS OF FEET)	PULSE RATE		RESPIRATORY RATE		PULMONARY VENTILATION (L./MIN. BTPS)		CO <sub>2</sub> OUTPUT (ML./MIN. STPD)		O <sub>2</sub> INTAKE (ML./MIN. STPD)		RESPIRATORY QUOTIENT	
		R.	Ex.	R.	Ex.	R.	Ex.	R.	Ex.	R.	Ex.	R.	Ex.
McNutt													
28	SL	74	108	11	21	6	22						
5	9	90	99	13	15	9	25	272	981	326	1145	.833	.857
10	14	91	130	14	17	10	29	257	916	332	1088	.774	.842
15	17	103	126	12	16	11	32	265	867	329	1019	.805	.852
19	18.5	108	134	15	13	14	34	284	924	351	1047	.810	.883
25	21	108	125	13	9	11	35	215	782	258	871	.832	.898
29	20		124	11	15	13	34	290	830	329	906	.881	.917
4	SL		110	9	9	8	27	194	824	233	934	.835	.882
Morris													
30	SL	67	112	14	11	7	16		660		878		
6	10	92	131	8	21	8	42	243	1363	291	1322	.834	1.032
11	15	100	142	13	18	12	43	297	1144	366	1171	.813	.977
16	17.5	115	141	10	19	10	53	210	1249	263	1198	.798	1.042
21	19	99		9	20	13	46	228	1058	263	1058	.868	1.000
26	21.5	111	138	9	14	13	46	270	845	307	864	.879	.978
30	21	102		10		13		224		271		.827	
5	SL	62	102	10	9	13	34	338	1103	362	1040	.934	1.060
Hertel													
29	SL	75		9	18	6	23	227	809	272	862	.832	.938
7	11	72	110	12	31	9	46	236	1087	285	1108	.828	.980
12	15.5	97	122	8	22	11	44	276	968	308	1005	.896	.964
17	18	88	130	11	37	11	61	230	988	277	1077	.831	.918
22	19.5	95		7	36	13	69	230	960	288	1022	.798	.939
27	22	82	110	24	41	19	61	273	784	321	838	.850	.934
31	20	102		18		23		286		298		.959	
Wilkins													
30	SL	72	130	14	22	8	35	247	1440	297	1538	.829	.936
8	12	89	130	13	24	11	34	270	1000	301	1055	.898	.948
13	16	100	134	18	25	10	39	204	929	253	1028	.804	.904
18	18	105	130	16	29	12	41	241	930	302	1012	.798	.920
23	20	97	136	16	27	10	50	186	972	233	1038	.796	.937
28	20	100	120	15	19	10	37	192	754	224	772	.856	.976
1	20		118		21		39		708		772		.918

illustrated by contrasting the resting studies on McNutt at 21,000 feet with those on Hertel at 22,000 feet. McNutt showed a relatively low ventilation (11 l./min.) which was associated with the extremely low alveolar  $pO_2$  of 30 mm. Hg,

an arterial  $pO_2$  of 29 mm. Hg, and an oxyhemoglobin saturation of 52 per cent. Hertel, although a thousand feet higher, by ventilating at the rate of 19 l./min.

TABLE 2

DATE	ARTERIAL $pCO_2$ (MM. HG) (EFFECTIVE ALVEOLAR $pCO_2$ )		EFFECTIVE ALVEOLAR $pO_2$ (MM. HG)		ARTERIAL $pO_2$ (MM. HG)		ALVEOLAR-ARTERIAL PRESSURE GRADIENT (MM. HG)	
	R.	Ex.	R.	Ex.	R.	Ex.	R.	Ex.
McNutt								
28	43	47			81	88	19	9
5		38		61	59	49		12
10	36	35	38	43	41	40	-3	3
15	23	31	45	38	35	33	10	5
19	23	27	41	38	38	26	3	12
25	26	22	30	37	29	31	1	6
29	23	24	38	38	36	33	2	5
4	30	28	116	119	93	99	23	20
Morris								
30	37	44			92	92		
6	28	32	67	70	65	62	2	8
11	28	26	47	53	45	42	2	11
16	22	23	44	49	41	37	3	12
21	21	24	43	42	39	32	4	10
26	20	20	36	39	31	30	5	9
30	17		41		41		0	
5	26	25	123	126	102	103	21	23
Hertel								
29	43	39	100	109	95	98	5	11
7	32	29	57	66	61	56	-4	10
12	27	25	49	52	52	41	-3	11
17	20	21	46	47	42	37	4	10
22	19	17	42	47	37	36	5	11
27	20	20	35	37	35	33	0	4
31	16		46		51		-5	
Wilkins								
30	44	45	99	103	92	97	7	6
8	31	31	57	59	56	50	1	9
13	26	30	45	43	45	40	0	3
18	23	24	41	44	39	36	2	8
23	25	22	33	41	35	30	-2	9
28	24		36		38		-2	
1		18		44		36		8

maintained an effective alveolar  $pO_2$  of 35 mm. and an oxyhemoglobin saturation of 66 per cent.

TABLE 3

DATE	COHb CONTENT (VOLUMES PER CENT)		O <sub>2</sub> Hb CONTENT (VOLUMES PER CENT)		O <sub>2</sub> Hb CAPACITY (VOLUMES PER CENT)		O <sub>2</sub> Hb SATURATION (PER CENT)	
	R.	Ex.	R.	Ex.	R.	Ex.	R.	Ex.
McNutt								
28	0.4	0.5	16.4	17.2	17.9	17.9		96
5	0.6	0.5	16.1	18.2	19.4	19.6	83	93
10	0.4	0.3	17.0	15.8	20.8	22.0	82	72
15	0.3	0.3	15.7	13.6	21.3	22.1	74	62
19	0.2	0.2	16.0	13.9	22.6	23.5	71	59
25	0.3	0.3	12.0	13.4	23.1	24.2	52	55
29	0.3	0.6	15.0	15.4	23.5	24.3	64	63
4	0.4	0.3	20.8	21.3	20.9	21.6	100	99
Morris								
30	0.7	0.7		20.5	21.1	21.6	93	95
6	0.8	0.6	18.7	20.0	21.5	22.8	87	88
11	0.5	0.6	18.9	19.4	23.5	24.1	80	80
16	0.5	0.5	20.1	17.5	25.2	26.2	80	67
21	0.4	0.3	18.2	15.3	23.4	26.7	78	57
26	0.4	0.6	15.6	16.6	25.4	27.0	61	62
30	0.5		18.8		24.9		76	
5	0.4	0.5	17.0	18.4	18.2	18.0	93	100
Hertel								
29	0.8	0.8	17.7	18.7	18.5	18.8	96	99
7	0.6	0.7	17.7	18.3	19.9	20.9	89	88
12	0.5	0.6	16.9	16.8	21.1	22.0	80	76
17	0.3	0.5	16.5	17.2	22.0	23.0	75	75
22	0.5	0.3	17.4	17.4	23.7	24.7	73	70
27	0.5	0.3	16.5	16.1	25.1	26.3	66	61
31	0.3		22.7		25.8		88	
Wilkins								
30	0.8	0.8	20.3	21.5	20.8	22.4	98	96
8	0.6	0.5	20.0	16.7	21.3	22.3	94	75
13	0.5	0.4	17.8	17.5	23.2	24.2	77	72
18	0.6	0.6	17.5	18.2	22.5	23.9	78	76
23	0.4	0.4	17.2	17.9	25.4	25.8	68	70
28	0.4	0.5	19.6		27.3		72	
1		0.3		20.2		28.8		71

Pulmonary ventilation remained elevated for at least four days in the two subjects studied upon return to sea level, a confirmation of the observation made by Schneider (27).

*Gas exchange.* The values for CO<sub>2</sub> output, O<sub>2</sub> intake and R.Q. shown in table 1 remained nearly constant during rest at all altitudes, indicating that there was

no appreciable effect of altitude on over-all bodily metabolism. This observation is in agreement with the findings of D'Angelo (28) and of Lewis et al. (29)

TABLE 4

DATE	ARTERIAL CO <sub>2</sub> CONTENT (VOLUMES PER CENT)		CO <sub>2</sub> CONTENT, FULLY OXYGENATED (pCO <sub>2</sub> = 40 MM. HG)		SERUM CO <sub>2</sub> CONTENT (VOLUMES PER CENT)		pH <sub>s</sub> (HENDERSON-HASSELBALCH)		pH <sub>s</sub> (GLASS ELECTRODE)	
	R.	Ex.	R.	Ex.	R.	Ex.	R.	Ex.	R.	Ex.
McNutt										
28	52.6	50.5	50.9	47.5	62.1	59.2	7.42	7.37	7.41	7.41
5	48.4	47.1	47.7	46.5	58.2	56.5	7.44	7.44	7.44	7.46
10	41.8	40.7	42.5	41.2	50.7	49.7	7.41	7.41	7.48	7.44
15	36.1	35.1	43.8	37.1	45.5	42.8	7.55	7.40	7.51	7.47
19	31.6	31.7	38.8	35.5	39.9	38.6	7.49	7.41	7.49	7.45
25	31.7	29.1	35.6	35.7	39.3	37.1	7.43	7.47	7.49	7.52
29	31.2	29.2	37.9	35.0	39.6	37.0	7.49	7.43	7.50	7.47
4	35.7	36.0	40.5	41.8	44.0	44.8	7.42	7.45	7.37	7.39
Morris										
30	45.0	43.7	46.0	40.5	54.9	52.0	7.44	7.33	7.42	7.37
6	41.6	36.2	47.6	38.7	52.5	44.7	7.53	7.40	7.47	7.41
11	37.1	29.7	42.3	35.4	47.1	37.7	7.48	7.41	7.52	7.45
16	31.6	26.1	40.5	32.7	41.6	33.7	7.53	7.41	7.54	7.45
21	29.3	25.9	37.8	30.8	37.7	33.0	7.50	7.38	7.53	7.44
26	27.7	22.5	36.2	30.1	36.2	29.5	7.50	7.41	7.52	7.50
30	25.7		37.4		34.5		7.55		7.55	
5	40.4	36.8	47.2	44.0	49.4	44.9	7.53	7.50	7.45	7.42
Hertel										
29	47.4	46.5	45.8	46.4	55.9	55.4	7.38	7.41	7.39	7.41
7	40.6	35.4	43.2	39.4	49.4	43.6	7.44	7.42	7.45	7.48
12	35.6	32.0	41.0	38.6	44.2	40.3	7.47	7.46	7.50	7.51
17	31.6	27.0	41.2	35.0	40.3	34.4	7.55	7.45	7.55	7.53
22	25.7	21.6	35.1	31.9	33.4	28.6	7.49	7.47	7.51	7.52
27	27.2	22.6	35.9	30.0	35.5	29.4	7.49	7.40	7.51	7.54
31	24.0		37.3		33.1		7.56		7.59	
Wilkins										
30	45.9	39.9	45.4		55.4		7.38		7.39	7.35
8	39.1	36.6	42.5	39.2	48.3	44.4	7.45	7.41	7.47	7.44
13	36.1	32.4	42.0	35.5	45.8	40.3	7.50	7.38	7.48	7.49
18	31.4	29.1	38.8	35.8	39.8	37.1	7.49	7.43	7.50	7.46
23	29.8	24.0	35.5	31.1	38.1	31.1	7.43	7.39	7.49	7.49
28	28.8		35.4		37.7		7.44		7.52	
1		25.3		36.5		35.1		7.53		7.53

and Dill (30), though not with those of Cook (31). The constancy of the respiratory quotient shows that these values were determined primarily by metabolic

TABLE 5

DATE	PLASMA									
	Hematocrit (per cent rbc)		Blood sugar (mgm./100 ml.)		Lactic acid (mgm./100 ml.)		Protein (gms./100 ml.)		Non- protein nitrogen (mgm./100 ml.)	Chloride (m-eq./l.)
	R.	Ex.	R.	Ex.	R.	Ex.	Kjeldahl	Sp.Gr.	Rest	Rest
McNutt										
28	39	39			12	18	6.1	5.9	39	106
5	46	49			15	24	5.8	5.7		105
10	48	51			21	33	6.6	5.8	44	104
15	53	54	98	93	21	30	6.1	6.4	46	104
19	54	56	119	101	22	31	6.3	6.0		
25	55	55	125	102	18	30	6.1	5.5	46	106
29	56	56	97	95	21	24	6.0	5.7	38	107
4					23	22	6.4		41	
Morris										
30	44	48			14	24	6.4	6.0	34	107
6	49	51			21	42				106
11	56	56			18	43	6.6		37	105
16	54	56	79	89	15	52	6.4	6.1		107
21	56	58	143	114	22	51	6.6	6.2	48	107
26	59	59	102	100	19	36	5.9	5.8	44	107
30	59		79		14		6.0	5.5	38	109
Hertel										
29	44	44			14	18	6.4	6.0	36	104
7	45	48			18	56	6.7	6.3		108
12	49	50			20	36	6.4	6.3		107
17	54	55	92	83	18	30	6.5	6.1	37	107
22	57	58	94	83	13	26	6.3	6.0	45	110
27	58	58	101	85	23		6.4	5.8	46	109
31	60		79		12	26	6.4	5.8	38	111
Wilkins										
30	46	48			20	51	5.7	5.7		110
8	48	51			18	30	6.2	5.5	35	
13	54	55			15	32	6.0	5.8	34	108
18	55	55	99	88	17	32	6.4	5.7	38	109
23	60	59	93	94	12	30	6.2	5.8	46	110
28	60		120		11		5.9	5.7	39	109
1			100		14		5.6	5.8	39	

requirements and not by transient alterations in ventilation. This finding lends increased significance to the higher than normal values for ventilation, which are thus shown to be representative of a fairly steady state.

During the standard work of 2530 ft. lbs./min. performed between 9000 and 20,000 feet, the  $O_2$  intake and  $CO_2$  output were also remarkably constant.

*Arterial and effective alveolar  $pCO_2$  and  $pO_2$*  (table 2). The observed drop in arterial  $pCO_2$  was an inevitable consequence of the sustained hyperventilation. In one instance (Hertel, 20,000 ft.) the arterial  $pCO_2$  reached the low value of 16 mm., but in none of the subjects was there any sign of impending tetany despite  $pH_s$  values of 7.5 or higher.

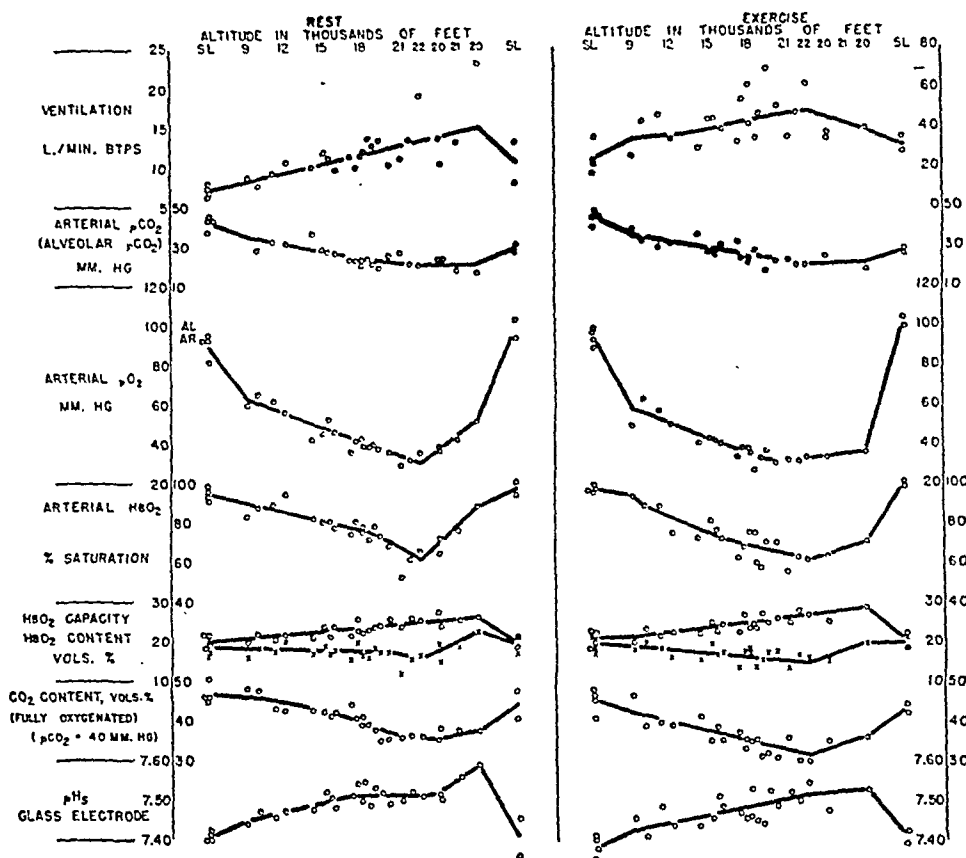


Fig. 2. Composite chart of selected data from all four subjects.

The level of the effective alveolar  $pO_2$  (20) was in general directly related to pulmonary ventilation, since the larger the respiratory minute volume, the more closely alveolar  $pO_2$  approaches the  $pO_2$  of inspired air (26).

*Alveolar-arterial  $pO_2$  gradient.* In general the difference in  $pO_2$  between alveolar air and arterial blood was less in the partially acclimatized subjects at altitude than it is in normal subjects at sea level (32). This is probably one of the physiological adaptations to low  $pO_2$  in the inspired air and may be related to an increase in both ventilation and pulmonary circulation. In previous unpublished studies we have noted that the alveolar-arterial oxygen pressure gradient decreases with voluntary hyperventilation during anoxia. The occasional finding of arterial oxygen pressures which were higher than the corresponding effective alveolar

oxygen pressures must be considered technical error in one or both figures since under the conditions of these experiments the directly determined arterial  $pO_2$  is only accurate to  $\pm 3$  mm. Hg and the calculated effective alveolar  $pO_2$  to  $\pm 4$  mm. Hg.

In keeping with previous findings, the alveolar-arterial gradients were larger during exercise. The unusually large gradients found in the two subjects studied on return to sea level may be attributed in part to technical error (since the direct arterial  $pO_2$  technic is less accurate for very high oxygen pressures), and in part to the increasing influence of venous admixture at high levels of oxygenation (32).

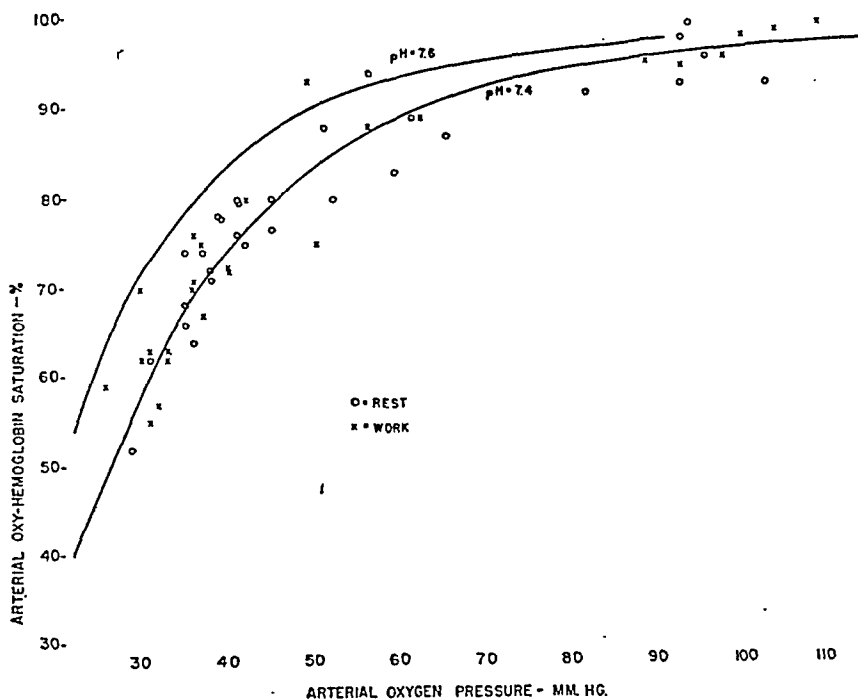


Fig. 3. Oxygen pressure saturation points determined on arterial blood, superimposed on standard oxyhemoglobin dissociation curves for pH 7.4 and 7.6.

*Oxyhemoglobin dissociation* (table 3, fig. 3). The oxyhemoglobin saturation decreased with increasing altitude as did the arterial  $pO_2$ , although the decrease was less marked than that of  $pO_2$  because of the shape of the oxyhemoglobin dissociation curve.

The scatter of the experimental points around the standard curve for  $pH = 7.4$  (12, 33) may be in part technical error but is significantly related to two biochemical factors. First, the individual pressure-saturation points correspond to arterial samples of different  $pH_s$  values and accordingly would not be expected to fall along the curve for any single  $pH_s$  value. Second, many individual points show a definite "shift to the right"; i.e., they fall to the right of the point to be expected for the  $pH_s$ ,  $pO_2$ , and saturation determined. This finding indicates an altered affinity of hemoglobin for oxygen at a given  $pH_s$  and is presumably an adaptation related to the prolonged alkalosis and hypoxia to which the subjects

were exposed. It is comparable to the changes found in 1935 by Keys et al. in studies of natives and temporary residents at high altitudes in Peru (34).<sup>5</sup>

*Oxyhemoglobin capacity and content.* The oxyhemoglobin capacity increased throughout the entire period at altitude to values which were high in comparison to sea level values, but which were not as high as those found in fully acclimatized subjects (35). Although total blood volume was not determined the constancy of the serum protein values indicates that the increase in capacity was probably due to an actual increase in hemoglobin rather than to hemoconcentration from fluid loss. Since these measurements were made after the subjects had been above sea level for 4-5 days, this observation does not conflict with the work of Asmussen and Nielsen (36) who found that the increase in hemoglobin which occurs during the first days at high altitude is due to hemoconcentration and not to an absolute increase in circulating red blood cells.

The oxygen content of the arterial blood remained almost constant from sea level up to 20,000 feet due to the fact that the increase in oxygen capacity compensated for the fall in oxygen saturation.

*Alkaline reserve, pH<sub>s</sub> and acid-base balance* (table 4). The CO<sub>2</sub> content of fully oxygenated blood at a pCO<sub>2</sub> of 40 mm. Hg (T40), which is a measure of the alkaline reserve, fell progressively with increasing altitude to approximately 75 per cent of the sea level value.

The values for pH<sub>s</sub> increased during the early part of the experiment and then leveled off at about 7.51. The highest single figure was 7.59, determined by glass electrode for Hertel at rest at 20,000 feet. The high pH<sub>s</sub> was associated with a very low pCO<sub>2</sub> (16 mm. Hg). These values are not to be considered as the normal response to altitude since the subject was apprehensive over the cardiac output procedure and was obviously over-breathing. These extreme changes in acid-base balance were not associated with signs of tetany.

Since pH<sub>s</sub> varies according to the Henderson-Hasselbalch formula,

$$\text{pH}_s = \text{pK}' + \log \frac{\text{BHCO}_3}{0.0301\text{pCO}_2}$$

restoration of the pH<sub>s</sub> in the presence of low pCO<sub>2</sub>, devolves upon the BHCO<sub>3</sub>. Although bicarbonate concentrations are not listed separately in the tables, they are practically the same as serum CO<sub>2</sub> content.<sup>6</sup> Like T40, serum CO<sub>2</sub> content showed a progressive fall to approximately 75 per cent of the sea level value.

In figure 4 the Henderson-Hasselbalch relationships for the resting bloods are plotted on triangular co-ordinates. The directly determined arterial pCO<sub>2</sub> values

<sup>5</sup> In the report of the 1922 Peruvian expedition (10) a "shift to the left" of the oxyhemoglobin dissociation curve was described. Possibly this finding resulted from Barcroft's use of pH<sub>s</sub> values significantly lower than those found by later investigators using more accurate methods. An error in this direction would cause an apparent shift to the left of the dissociation curve.

<sup>6</sup> Total serum CO<sub>2</sub> content includes both bicarbonate and dissolved CO<sub>2</sub>, but the ratio of concentrations is normally about 20:1. Dissolved CO<sub>2</sub>, which varies with pCO<sub>2</sub>, thus makes a relatively insignificant contribution to total serum CO<sub>2</sub> while bicarbonate makes the major contribution.

and the directly determined glass electrode  $pH_s$  values were used. Normal sea level points determined before the experiment was started appear in the upper left hand part of the chart. As the altitude increased the points moved down and to the right, then down between the  $pH_s$  7.5 and  $pH_s$  7.6 lines. If arterial  $pCO_2$  is considered fixed by the obligatory hyperventilation at altitude, the distribution of the points shows the degree to which the lowering of plasma bicarbo-

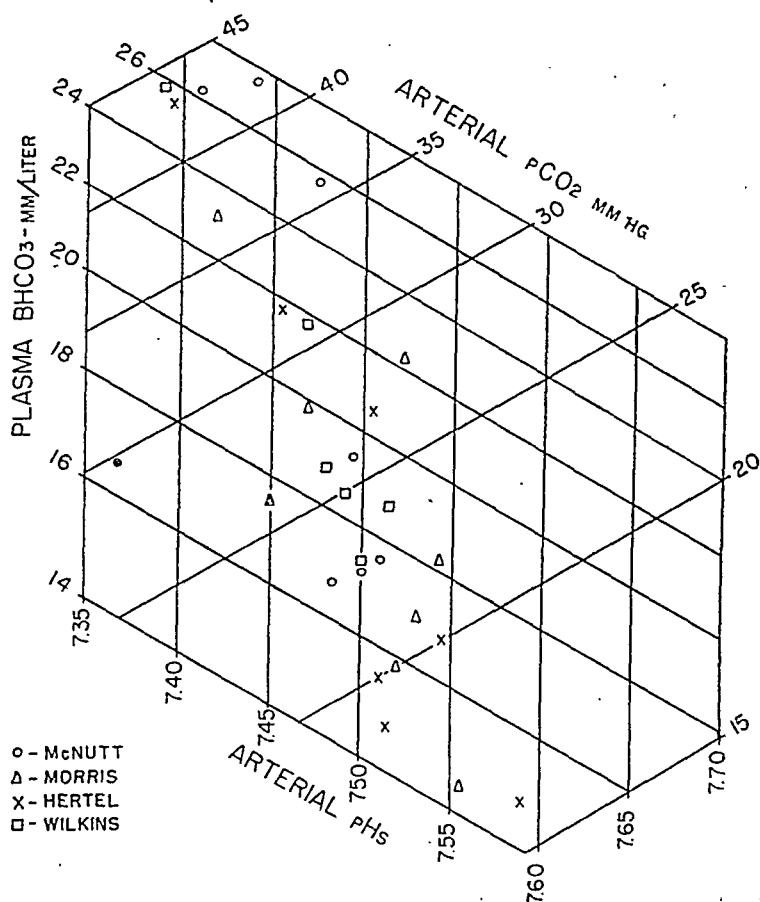


Fig. 4. Acid-base relationships plotted on triangular co-ordinates. Data obtained from resting bloods on all four subjects. The shaded points were obtained after return to sea level.

nate limited the rise in  $pH_s$ . The two points determined after return to sea level (cross hatched) showed normal  $pH_s$  values, but  $BHCO_3$  and  $pCO_2$  were still low by sea level standards.

In this experiment there was little change in the concentration of negative ions other than bicarbonate: chloride, although slightly elevated, showed a definite change from the sea level value in only one subject; protein showed no change; lactate showed a slight rise above the sea level value in three of the four subjects. Bicarbonate decreased approximately 4.5 m.-eq./l. which is probably more than the increase in other negative ions. If the sum of all negative ions including bi-

carbonate decreased slightly, a corresponding decrease in positive ion concentration must also have occurred in order to restore ionic equilibrium. Although our data are not complete enough to establish these changes with certainty, they are consistent with the findings of Dill et al. (12).

*Cardiac output and arterio-venous oxygen difference.* Of the two cardiac output determinations which were performed, the one on McNutt at 20,000 feet was the more satisfactory since the subject was not far from a basal state and the technical aspects of the dye injection and multiple arterial sampling went smoothly.<sup>7</sup> The value obtained was 9.8 l./min., which, with an oxygen intake of 329 ml./min., indicates an arterio-venous oxygen difference of 3.35 vols. per cent. Since the arterial oxyhemoglobin content was 14.95 vols. per cent, the mixed venous blood must have contained 14.95 - 3.35 or 11.6 vols. per cent. The oxyhemoglobin saturations of arterial and mixed venous blood were 64 and 49 per cent respectively. The arterio-venous difference was thus 15 per cent in saturation or 8 mm. Hg in oxygen pressure (as read from the oxyhemoglobin dissociation curve).

The cardiac output determination on Hertel at 20,000 feet (less satisfactory because the subject was excited) showed the minute volume of blood flow to be 16.8 l./min. The calculated arterio-venous difference was therefore 1.8 vols. per cent in saturation and 9 mm. Hg in oxygen pressure. Since Hertel's level of oxygenation was higher than that of McNutt, his arterio-venous difference, although lower in per cent saturation, was actually larger in terms of oxygen pressure. If the normal resting cardiac output is considered to be 5 l./m. at sea level, the figure for McNutt was almost twice, and that for Hertel more than three times normal.

*Effect of exercise.* The work load at sea level was different for each individual and unfortunately these data cannot be compared with the data collected during the standard work at altitude.

The work performed at altitude required an oxygen intake of slightly more than three times the resting rate and was associated with an increase in pulmonary ventilation to three or four times the resting rate (table 1). As was the case at rest, oxygen consumption during standard work was not significantly changed as the altitude increased. The highest ventilation during work was Hertel's at 19,500 feet, which reached 69 l./m. BTPS at a respiratory rate of 36 per minute. The highest pulse recorded during work was 142 for Morris at 15,000 feet.

The arterial  $pO_2$  during work at sea level remained the same as, or actually increased above, the resting level, but at altitude the arterial  $pO_2$  during work was usually several millimeters Hg below the resting level. At altitude the alveolar-arterial oxygen pressure gradient was larger during work than while at rest, in keeping with earlier experience (32). Oxyhemoglobin content usually remained constant and oxyhemoglobin capacity increased slightly with the net result that oxyhemoglobin saturation usually dropped during exercise.  $CO_2$  content dropped probably because of the increase in lactate ion. At the level of work studied there was no evidence of the decrease in lactic acid production or

<sup>7</sup> The determinations were made by Dr. John W. Remington.

tolerance at altitude found by Edwards (37). The  $pH_s$  usually, though not invariably, decreased slightly during work.

**DISCUSSION.** The data recorded in this paper fit into the concept advanced by Barcroft (5) that acclimatization to high altitude consists of a series of integrated adaptations which tend to restore tissue oxygen pressure toward normal sea level values in spite of lowered oxygen pressure in the atmosphere. Since at any given altitude the  $pO_2$  of the inspired air is fixed, tissue  $pO_2$  can be restored toward normal only by diminishing the drop in  $pO_2$  between inspired air and tissues. Accordingly, in order to analyze the changes occurring in the oxygen transport system as a result of acclimatization, we shall examine the  $pO_2$  gradients which occur at successive stages along the route of oxygen transport.

From the data at hand we can compare the  $pO_2$  gradients from inspired air to alveolar air, and from alveolar air to arterial blood in acclimatized and unacclimatized individuals. On theoretical grounds we should like to continue the analysis to include the  $pO_2$  gradients from arterial blood to capillary blood, and from capillary blood to tissue cells. Unfortunately the gradient from capillary blood to tissue cells is beyond hope of quantitative approximation from the data at hand, but the arterial-capillary  $pO_2$  gradient can be estimated with enough accuracy to be very helpful in evaluating the relative importance of certain circulatory adaptations to high altitude.

In order to obtain a single figure for the  $pO_2$  gradient between arterial blood and capillary blood it is necessary to assign an average or mean value to a function which is in fact constantly changing. The arterial  $pO_2$  is the same for all the capillaries of the body and is readily determined by direct analysis; the capillary  $pO_2$ , however, varies widely, and it is this end of the gradient for which a theoretical average value must be obtained. The concept of a mean capillary  $pO_2$  was first introduced by Bohr (38), who estimated this value for blood in the pulmonary capillaries by a graphic integration method. Barcroft (5) expanded the idea and applied it to the capillaries of the greater circulation. Houston (39) has recently shown graphically how this expanded concept can be used to evaluate the relative importance of factors involved in acclimatization to high altitude.

The mean capillary  $pO_2$  may be defined as that oxygen pressure which, if it prevailed throughout the entire length of all the capillaries of the body, would not alter the quantity of oxygen diffusing from capillaries to tissues from the quantity diffusing under actual physiological conditions. The method for calculating mean capillary  $pO_2$  is a modification of Bohr's graphic integration procedure<sup>8</sup> and depends upon the basic principle that the rate at which oxygen diffuses across a membrane (in this case the capillary wall and tissue fluids) is directly proportional to the  $pO_2$  gradient between the two sides of the membrane. In calculating this value the necessary data are the arterial  $pO_2$ , the mixed venous  $pO_2$ , and the mean

<sup>8</sup> During moderate and severe anoxia the results obtained by the lengthy integration procedure are almost identical to those obtained by the simple approximation suggested by Barcroft:

$$\text{Mean capillary } pO_2 = \text{Venous } pO_2 + \frac{\text{Arterial } pO_2 - \text{Venous } pO_2}{3}$$

tissue  $pO_2$ . The arterial  $pO_2$  is determined from the brachial artery sample. The mixed venous  $pO_2$  refers to the blood entering the right heart which is a mixture of venous blood from all parts of the body.<sup>9</sup> In the analysis which follows, mixed venous  $pO_2$  is calculated from the cardiac output and the oxygen consumption according to the Fick relationship. Mean tissue  $pO_2$  has been arbitrarily considered to lie half way between zero and mixed venous  $pO_2$ . At high altitude this arbitrary choice introduces no significant error in the calculation of mean capillary  $pO_2$ . For sea level conditions (arterial  $pO_2$  of 95 mm. Hg and mixed venous  $pO_2$  of 35 mm. Hg), the exact value of mean tissue  $pO_2$  is a more important factor: mean capillary  $pO_2$  will vary by 7 mm. Hg if mean tissue  $pO_2$  ranges from 1 mm. to 34 mm. For an assumed mean tissue  $pO_2$  of 17 mm. (midway between zero and mixed venous  $pO_2$ ) the mean capillary  $pO_2$  is 47 mm.

Let us proceed to an examination of the  $pO_2$  gradients occurring at each stage in the oxygen transport system before and after acclimatization. In the following analysis we shall use data on Hertel at sea level and on McNutt at 20,000 feet

TABLE 6

	INSPIRATORY $pO_2$ , MM. HG*	ALVEOLAR $pO_2$ , MM. HG	ARTERIAL $pO_2$ , MM. HG	MEAN CAPILLARY $pO_2$ , MM. HG
Sea Level Hertel.....	152	100	95	47
Gradient.....	52	5	48	
20,000 ft. McNutt.....	64	38	36	32
Gradient.....	26	2	4	

\* At 37 degrees C. and complete saturation with water vapor, and corrected for the difference in volume between inspired and expired air.

because the most satisfactory determinations were obtained in these two instances. The data are summarized in table 6.

The drop in  $pO_2$  between inspired air and alveolar air was 26 mm. Hg less at altitude than at sea level (26 mm. instead of 52 mm.). The mechanism by which this diminution was accomplished was increased ventilation (Barcroft's first step). For normal individuals doubling the ventilation approximately halves the  $pO_2$  gradient between inspired air and alveolar air (26); in this case the ventilation increased from 6 to 13 l./min. and the gradient was halved.

The  $pO_2$  gradient between alveolar air and arterial blood was 3 mm. less at 20,000 feet than at sea level (2 mm. instead of 5 mm.). At 20,000 feet this gradient was due almost entirely to the diffusion resistance of the pulmonary membrane, and the diffusion constant of the lung, as calculated by the method of Lilienthal et al. (32), is found to be 70. For sea level conditions the alveolar-

<sup>9</sup> Venous blood leaving the capillaries varies from capillary to capillary and from moment to moment because of various changing factors which include blood flow, proximity of patent capillaries, cellular metabolism, diffusion characteristics, etc. The blood in the right auricle is a mixture of the venous bloods from all the capillaries of the body and thus provides a physiologically integrated mean venous  $pO_2$ .

arterial  $pO_2$  gradient must be differently interpreted, and on this account the determinations which were adequate for calculating the diffusion constant at altitude were insufficient at sea level. Previous studies have shown, however, that the diffusion constant ranges between 20 and 30 under normal conditions. The increased value at altitude probably resulted from increased perfusion of alveolar capillaries and increased alveolar ventilation. Both of these factors increase the effective diffusing surface in the lung and would therefore be expected to diminish diffusion resistance and increase the diffusion constant of the lung.

TABLE 7

	ARTERIO-VENOUS DIFFERENCE			ART. $pO_2$ MM. HG	MEAN CAP. $pO_2$ MM. HG	GRADIENT MM. HG
	ml. $O_2$ /l. blood	$O_2$ Hb Sat. %	$pO_2$ mm. Hg			
96% Arterial $O_2$ Hb Saturation (Hertel—Sea level)	$\frac{272}{5} = 54$	$\frac{5.4}{18.5} = 29$	60	95	47	48
64% Arterial $O_2$ Hb Saturation (McNutt—20,000 ft.)						
A. Assumed: cardiac output and $O_2$ Hb capacity as for Hertel at sea level	$\frac{329^*}{5} = 66$	$\frac{6.6}{18.5} = 36$	17	36	26	10
B. Cardiac output as found: $O_2$ Hb capacity as for Hertel at sea level	$\frac{329}{9.8} = 34$	$\frac{3.4}{18.5} = 18$	10	36	31	5
C. Cardiac output and $O_2$ Hb capacity as found	$\frac{329}{9.8} = 34$	$\frac{3.4}{23.5} = 15$	8	36	32	4

A. Illustration of the effect of the shape of the oxyhemoglobin dissociation curve upon the gradient between arterial  $pO_2$  and mean capillary  $pO_2$ .

B. Illustration of the additional effect of an increase in cardiac output.

C. Illustration of the additional effect of an increase in  $O_2$ Hb capacity.

\* For purposes of isolating the factors under A, B, and C it would have been desirable for the oxygen consumption of McNutt (329 ml./min.) to be the same as that of Hertel (272 ml./min.). However, the effect upon the gradient between arterial  $pO_2$  and mean cap.  $pO_2$  is minor.

The estimated drop between arterial  $pO_2$  and mean capillary  $pO_2$  was 48 mm. at sea level but only 4 mm. at altitude. In table 7 this gradient is subdivided to show the part played by each of the three major factors involved. The factor most largely responsible for the smaller gradient at altitude as compared to sea level is directly related to the characteristics of hemoglobin (table 7, A). The use of the mean capillary  $pO_2$  makes possible a more accurate estimation of the physiological significance of this factor than has previously been possible. The shape of the oxyhemoglobin dissociation curve is such that at sea level (high  $pO_2$ )

a relatively large pressure drop is required for a given decrease in saturation (fig. 5). For the steeper portion of the curve at high altitude, however, the same decrease in saturation is accomplished by a much smaller drop in  $pO_2$ . In the illustrative figure, a decrease of 25 per cent in saturation is seen to require a  $pO_2$  drop at sea level more than four times as great as that required at altitude. Mean capillary  $pO_2$  lies between arterial and venous  $pO_2$ , and factors which reduce the arterio-venous difference in  $pO_2$  likewise reduce the gradient between arterial

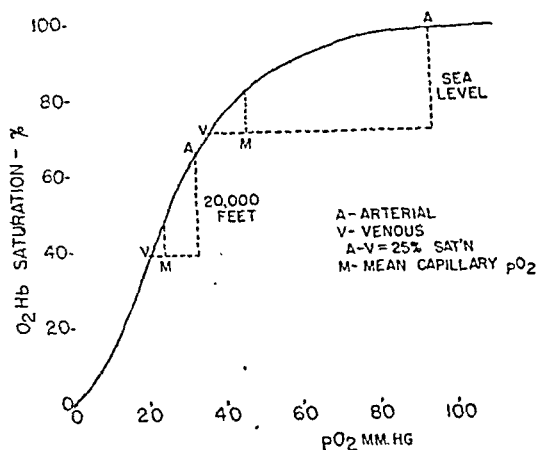


Fig. 5

Fig. 5. Standard oxyhemoglobin dissociation curve for pH 7.4, showing how the level of oxygenation affects the  $pO_2$  drop required for an arterio-venous difference of 25 per cent saturation.

Fig. 6. The oxygen pressure gradients at various stages of the oxygen transport system. Upper curve from data obtained on Hertel at sea level; lower curve from McNutt at 20,000 feet.

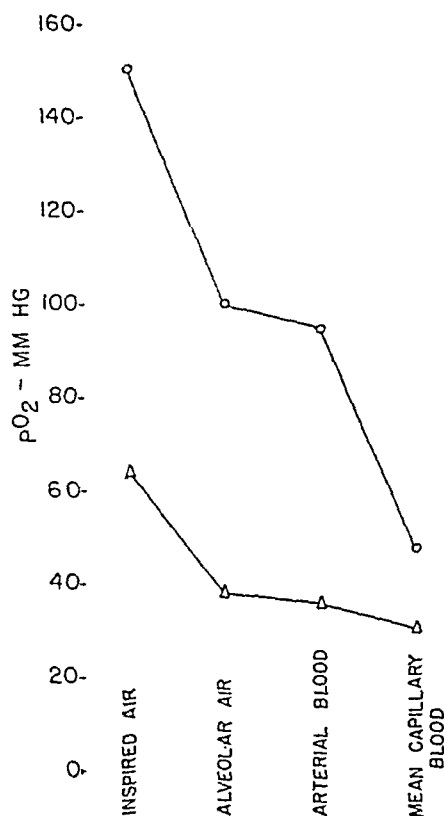


Fig. 6

$pO_2$  and mean capillary  $pO_2$ . Specifically (table 7, A), this gradient was  $95 - 47$  or  $48$  mm. when the arterial saturation was 96 per cent, whereas when the arterial saturation was 64 per cent, the gradient would have been 10 mm. ( $36 - 26$ ) had other adaptations not lowered it further. Due therefore to the characteristics of the dissociation curve alone, the gradient between arterial and mean capillary  $pO_2$  was reduced by 38 mm. ( $48 - 10$ ) at altitude.

The second factor which reduced the gradient was the increase in cardiac out-

put (Barcroft (5) step three) which occurred at altitude (table 7, B). If arterial  $pO_2$  and oxygen consumption are fixed, the increased cardiac output, by decreasing A-V difference, raises the venous  $pO_2$ , thereby raising mean capillary  $pO_2$ . The net result of the increase in cardiac output was therefore a further lowering of the gradient between arterial  $pO_2$  and mean capillary  $pO_2$  of 5 mm. In the few measurements previously reported (40, 41) the cardiac output was found to return nearly to normal as acclimatization progressed.

The third and least important factor was the increase in the oxygen carrying power of the blood (Barcroft's step two), attendant upon the polycythemic response to altitude. Although this adaptation is commonly considered a major factor in acclimatization, it may be seen from table 7, C, that an increase in capacity from 18.5 to 23.5 vols. per cent further reduced the  $pO_2$  gradient by only 1 mm. (from 5 to 4 mm.). Even if the oxyhemoglobin capacity had increased to 26 vols. per cent, the gradient would have been reduced by only 2 mm. This interesting observation indicates (as was suggested in 1928 by Campbell (42) and found in 1932 by Hurtado (43)) that polycythemia is not essential for acclimatization, but is in fact only one small element in the remarkably flexible adaptive process.

The three stages which we have analyzed in the oxygen transport system are summarized in figure 6. When the data at altitude are compared to those at sea level, it is clear that the most marked reduction in  $pO_2$  gradient occurred between arterial and mean capillary blood, a reduction due primarily to the inherent and virtually unchangeable characteristics of hemoglobin. Increase in pulmonary ventilation, the most rapidly and easily accomplished adaptation to altitude, caused the next most marked reduction in gradient (inspiratory  $pO_2$  to alveolar  $pO_2$ ). The changes in cardiac output, in the oxygen capacity of the blood, and in the diffusion characteristics of the "pulmonary membrane" were quantitatively less effective. All of the changes taken together sustained tissue oxygenation at a remarkably high level, by lowering the gradient between inspired  $pO_2$  and mean capillary  $pO_2$  from 105 mm. at sea level to 32 mm. at 20,000 feet in the example shown. In spite of this striking change, mean capillary  $pO_2$  was 15 mm. lower at 20,000 feet than at sea level (32 instead of 47 mm.), indicating that compensation was not complete. It is not surprising, therefore, that signs of tissue anoxia in all four subjects were easily detected by the normal observers, even though the subjects felt well and considered themselves in good condition.

Although there were evidences of tissue anoxia, the oxygen consumption during rest and during standard work remained the same at altitude as at sea level. In other words, there was no evidence that cellular metabolism decreased as a part of the acclimatization process. It thus appears that cellular function was impaired by low  $pO_2$  even though the amount of oxygen used by the cells remained normal.

The efficiency of cellular metabolism at a given  $pO_2$  involves the various enzyme systems, and it is conceivable that substances such as cytochrome C may be capable of restoring cellular function toward normal even at low oxygen pressures.

The same pulmonary and circulatory factors which help to sustain tissue  $pO_2$

cause a decrease in tissue  $p\text{CO}_2$ . The effects upon  $p\text{O}_2$  and  $p\text{CO}_2$  are opposite, but the mechanisms involved are the same: the partial pressure of the gas in the tissues is made to approach the partial pressure of that gas in the inspired air. While there may be direct effects of low  $p\text{CO}_2$  upon cellular metabolism, we shall consider  $p\text{CO}_2$  only in relation to the pH of the blood.

At sea level the maintenance of a stable  $\text{pH}_s$  is facilitated by the rapid adjustment of  $p\text{CO}_2$  which is accomplished by changes in pulmonary ventilation. At high altitude, however, the anoxic stimulus to ventilation is so urgent that  $p\text{CO}_2$  is invariably low. It is no longer a labile factor available as a buffer against change in  $\text{pH}_s$ ; on the contrary, it is the cause of the rise in  $\text{pH}_s$  or respiratory alkalosis which was a prominent feature in our acclimatizing subjects.

In contrast to  $p\text{CO}_2$  which is controlled by pulmonary ventilation,  $\text{BHCO}_3$  is dependent upon the concentration of those positive ions remaining uncovered by other negative ions. If available base, or alkaline reserve, increases,  $\text{CO}_2$ , which is always available in abundance, balances it in the form of bicarbonate, thus restoring ionic equilibrium. If the alkaline reserve decreases, the bicarbonate ion is immediately lowered by rapid elimination through the lungs in the form of  $\text{CO}_2$ . The bicarbonate concentration is thus governed by the other serum electrolytes (chloride, protein, lactate, total base, etc.). Changes in bicarbonate and in the other electrolytes may be considered secondary factors in acclimatization; indirectly they are adaptations to low  $p\text{CO}_2$ .

Since the reduction in bicarbonate which occurs during acclimatization can proceed no faster than the accompanying changes in electrolyte concentration and since electrolyte concentration depends upon the rate of excretion by the kidneys, it appears that kidney function sets the pace in restoring  $\text{pH}_s$  to normal. At sea level the task of buffering the blood against change in  $\text{pH}_s$  is divided between the lungs and the kidneys, but at high altitude the obligatory increase in ventilation causes the lungs to hinder rather than to help in restoring  $\text{pH}_s$  toward normal. Accordingly the buffering function falls more heavily upon the kidneys, and it is on this account that restoration of  $\text{pH}_s$  values toward normal takes a prolonged period of time. The subjects of this experiment still showed high  $\text{pH}_s$  values after one month of exposure to increasing altitude.

On return to sea level the anoxic stimulus to ventilation is immediately relieved, the lungs resume their accustomed rôle in the buffering mechanism, and  $\text{pH}_s$  returns to normal. The acclimatized subject still has a low  $\text{BHCO}_3$ , however, and in order to maintain a normal  $\text{BHCO}_3/\text{H}_2\text{CO}_3$  ratio the  $\text{H}_2\text{CO}_3$  must remain low. But low  $\text{H}_2\text{CO}_3$  depends upon low  $p\text{CO}_2$  which in turn requires a high minute volume of ventilation. Accordingly, increased pulmonary ventilation continues until the kidneys have made electrolyte adjustments which permit  $\text{BHCO}_3$  to return to its normal sea level value. These readjustments again require a considerable period of time.

Although the data collected in this experiment do not permit an analysis of the control of breathing during oxygen deficiency, they are consistent in all major respects with the findings of Bjurstedt in dogs (44). As the altitude increased the subjects showed varying degrees of hyperventilation with respiratory alkalo-

sis. For at least the early part of their period of residence at high altitude ventilation was probably sustained largely by chemoreflex drive from the carotid body. The chemosensitive cells of the respiratory center in the brain, which respond to increased acidity, were probably inactive due to missing stimulation. On return to sea level the anoxic stimulus to chemoreflex drive was immediately relieved, but control was promptly taken over by the respiratory center. Of this there can be little doubt because hyperventilation was maintained, in the absence of anoxia, during a transition period of at least four days during which reduction in ventilation would have occasioned acidosis.

Exercise at sea level, by increasing oxygen consumption, tends to lower tissue  $pO_2$ . This tendency is combatted by increased ventilation (which raises alveolar  $pO_2$ ), by an increase in the diffusion constant of the lung (which minimizes the drop in oxygen pressure between the alveoli and the arterial blood), and by an increase in cardiac output and in the oxygen carrying capacity of the blood (which minimizes the gradient between arterial  $pO_2$  and mean capillary  $pO_2$ ). During exercise at sea level tissue  $pO_2$  is thus prevented from falling by mechanisms comparable to those by which tissue  $pO_2$  is restored toward the normal during rest at high altitude. Exercise at high altitude imposes a double stress which can be met only by extremely vigorous ventilatory and circulatory responses.

The characteristics of hemoglobin and the minute volume of ventilation have been shown to be of preponderant importance in the process of acclimatization to high altitude. Since the benefits related to hemoglobin dissociation are available to the unacclimatized man, one may well ask why voluntary hyperventilation might not sustain the unacclimatized individual at high altitude. Many workers have shown this to be possible, and in additional studies at this laboratory a subject remained in good condition by this means for four hours above 20,000 feet. Arterial  $pO_2$  and  $pCO_2$  values were similar to those of the acclimatized subjects, but there was no tendency for the increased breathing to become automatic. Quite the contrary, every breath required conscious effort and attention. This subject lacked the adaptations to low  $pCO_2$  which made adequate automatic breathing possible for the acclimatized subject.

#### SUMMARY AND CONCLUSIONS

Detailed studies of the respiratory and circulatory changes which occur during the process of acclimatization to oxygen lack were made on four men exposed to gradually increasing simulated altitude during one month in a low pressure chamber.

The data obtained strengthen the concept that acclimatization consists of a series of integrated adaptations which tend to restore the oxygen pressure of the tissues toward normal sea level values despite the lowered  $pO_2$  of the atmosphere.

The transfer of oxygen from inspired air to tissue cells can be conveniently divided into several stages which together comprise the oxygen transport system. A theoretical mean value for the capillary oxygen pressure has been intro-

duced to make possible a more quantitative evaluation of circulatory factors than heretofore possible.

The reduction in the  $pO_2$  gradient between inspired air and mean capillary blood was due mostly to the shape of the oxyhemoglobin dissociation curve and to an increase in pulmonary ventilation; increase in cardiac output, increase in the diffusion constant of the lung, and increase in oxyhemoglobin capacity were less important factors.

The same pulmonary and circulatory changes which caused an increase in  $pO_2$  necessarily caused a decrease in  $pCO_2$ , and an initial effect of the decrease in  $pCO_2$  was an increase in the alkalinity of the blood. Further changes occurred, as acclimatization progressed, to counteract this respiratory alkalosis. The fall in blood bicarbonate reflected the extent of these changes which included a net increase in the other negative ions and probably a net decrease in the positive ions. These changes comprised secondary factors in acclimatization.

There was no evidence that cellular metabolism decreased as part of the acclimatization process, since the oxygen consumption remained the same at altitude as at sea level, both during rest and during standard work. Since clinical evidence indicated that the subjects were moderately anoxic, it appears that cellular function was impaired by low  $pO_2$ , even though the amount of oxygen used by the cells remained normal.

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# EFFECT OF ADRENOCORTICAL AND SYMPATHICO-ADRENAL FACTORS ON THE CARDIOVASCULAR SENSITIVITY TO POTASSIUM IN THE RAT<sup>1</sup>

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Despite the fact that the rat given ergotamine and the animal bearing autoplasmic transplants of adrenal cortex differ in mode of regulation of extracellular potassium (1), they have in common the phenomenon of adrenalin reversal (2, 3).

This point is of interest, since the rat bearing adrenocortical grafts is subject to a variable degree of cortical deficiency (4, 5, 6) with, however, little apparent disturbance of sympathetic function. In the rat treated with ergotamine, on the other hand, sympathetic excitatory activity is largely if not entirely annulled; there is, however, no apparent deficiency in cortical function.

In view of these circumstances, the similarity of vascular reactions to small amounts of adrenalin is rather noteworthy, since it might be assumed that the diversity in potassium balance (1) and control might suggest a dissimilar pattern of response.

**METHODS.** The following experiments were designed along qualitative rather than quantitative lines to determine whether significant changes might be observed in the tolerance to circulating K following radical disturbances of sympathetic function by (a) removal of the adrenal medulla and/or by (b) blocking of sympathetic excitor mechanisms by a sympatholytic agent. Normal rats, rats with autoplasmic adrenocortical transplants and animals treated with just sufficient ergotamine tartrate to produce adrenalin reversal and presumably adequate to suppress other sympathetic excitatory mechanisms (7, 8, 9, 10) were compared with regard to: 1, the tolerance to repeated intravenous injections of KCl, that is, the load required to produce a threatened circulatory collapse; 2, the level of plasma potassium attained at this time, i.e. (1); 3, the effect of mild histamine shock on the response to the intravenous injections of KCl.

The operative and technical procedures were the same as those previously described (11). Adult male rats (300 to 400 grams) were used. All rats with adrenocortical transplants (ACT-rats) were operated at 2 to 3 months of age and used not earlier than 3 months after operation.

All animals treated with ergotamine (ET-rats) received 0.5 mgm. per 100 grams body weight of Gynergen<sup>2</sup> either intraperitoneally or intravenously.

<sup>1</sup> This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

<sup>2</sup> Ergotamine tartrate, kindly supplied by Sandoz Chemical Works, Inc.

Histamine (ergamine phosphate)<sup>3</sup> in the amount of 10 mgm. per 100 grams body weight was administered intraperitoneally. As a routine only aqueous rather than saline solutions were used to avoid any effect of the Na-ion.

In the experiments on evaluating the sensitivity to intravenously administered KCl, carotid blood pressure changes were recorded by the mercury manometer under urethane anesthesia. Heparin was given by vein to prevent clotting. This method sufficed to demonstrate differences between the three types of animals, using as an end-point a marked cardiac depression with threatened circulatory collapse. This followed upon a series of injections of 0.05 ml. per 100 grams, given at 4 to 5 minute intervals. The rate of injection was 0.02 ml. per second. A 2 per cent solution of KCl was used throughout. With this concentration the initial responses were more consistent within each group, and the time and number of injections required to attain toxic levels fell within practicable limits.

RESULTS. 1. *Comparison of the tolerance to KCl administered intravenously.* Preliminary studies indicated that ACT-rats were much more sensitive to injected potassium than normal controls. Of 19 normal rats 13 survived repeated injections amounting to 7 to 8 mgm. K per 100 grams, whereas only 2 of the 13 ACT-rats survived after receiving a total of 1 to 4 mgm. K per 100 grams. Similar observations were not possible on rats treated with ergotamine in view of the variety of experimental procedures in this group.

To test this suspected disparity between the untreated rat, the ergotamine-treated rat and the rat with a deficiency in both adrenal hormones, 5 to 10 of each group were injected repeatedly with the standard dosage of KCl (see methods) to determine the respective, tolerated loads.

In normal rats, the first few injections as a rule produced double-peaked pressor effects separated by a slight fall. After 10 to 15 injections the insignificant depressor effect began to increase proportionately to the point of circulatory collapse. From 20 to 28 injections were required to produce a 50 to 70 per cent fall in blood pressure with a significantly delayed onset of recovery; in one case 40 injections were given. Recovery usually took place spontaneously.

In ACT-rats the initial response was usually one of simple depression followed by secondary compensatory rise. After relatively few injections (2 to 8) the fall in mean pressure at the last injection increased to over 70 per cent, followed within 1 to 2 minutes by an abrupt compensatory rise. In these rats the change from an innocuous fall to a pre-terminal collapse was unpredictable. Artificial respiration and massage of the heart was generally required to insure recovery.

In the ET-rats, as in rats with no adrenal medullae, a profound fall in pressure occurred with the 3rd to 8th injections but unlike the demedullated group, compensatory pressor effects were conspicuously reduced or absent. In addition, the last 20 to 30 per cent of the return to the original level of blood pressure was considerably delayed. This abrupt change in sensitivity showed an unex

<sup>3</sup> Burroughs Wellcome & Co.

pected but close similarity to that exhibited by the demedullated rat except for the delayed completion of recovery and the reduction or absence of a compensatory rise in pressure. Again thoracic massage was necessary to initiate recovery of the circulation and counteract the respiratory depression. The comparative data on these experiments is summarized in table 1.

2. *Comparison of the plasma K after a series of intravenous injections of KCl.* When the cardiovascular system became highly sensitive to a further increase

TABLE 1

*Comparison of the amount of intravenously injected KCl in normal, ACT- and ET-rats to produce a threatened circulatory collapse*

CLASS NO.	NORMAL RATS (6)		ET-RATS (7)		ACT-RATS (10)	
	Average	Range	Average	Range	Average	Range
Initial mean pressure	72	55-90	70	60-90	61	50-91
Number of KCl injections	27	20-40	5	3-8	4	2-8
mm. Hg fall on last injection,	46	36-60	71	55-88	50	31-66
Duration, sec.		50-60		65-140		57-220
Recovery characteristics	Spontaneous, compensatory rise marked		Assisted, compensatory rise reduced or absent, slow completion		Assisted, compensatory rise marked	

TABLE 2

*Comparison of the amount of K injected intravenously and the plasma K level attained in normal, ACT- and ET-rats*

CLASS AND NUMBER OF RATS USED	AMOUNT OF K INJECTED (MG. PER 100 GRAMS)		PLASMA K (MG. PER 100 ML.)	
	Average	Range	Average	Range
Normal rats, (6).....	13.6 $\pm$ 3.1	10 -20	29.8 $\pm$ 3.03	25.7-34.3
ET-rats, (7).....	2.5 $\pm$ 1.02	1.5- 3.5	23.8 $\pm$ 1.7	21.5-26.0
ACT-rats, (10).....	2.0 $\pm$ 0.85	1.0- 4.0	28.5 $\pm$ 2.22	24.6-31.6

of circulating K, the plasma K level was elevated about equally in the normal and in the demedullated rats. The critical level, however, was significantly lower in the ET-rat.

In table 2 are presented the comparative amounts of potassium injected and the plasma K values found after the last injections for each of the three groups of animals.

For comparative purposes plasma K was determined for 3 normal rats after

4, 6 and 7 injections of the standard dose of KCl, and values of 17.5, 20.2 and 20.6 mgm. per cent respectively were obtained, showing that at first the shift of excess K to tissue spaces was more rapid than the rate of increase by injection (normal resting value, 21.3 mgm. per cent). Hence, to some extent the ET-rat, unlike the ACT-rat, was less able to prevent a rise in circulating K than the normal but in addition showed an unusual sensitivity to this cation. In both groups the augmentation of the depressor response with 3 to 6 injections was like the normal response to 15 to 20 injections. Apparently after 15 to 20 doses of KCl the plasma K in the normal rat began to rise significantly; the value obtained for one case after 20 injections was 25.7 mgm. per cent whereas the rat receiving 40 injections had the highest value of the group, namely 34.4 mgm.

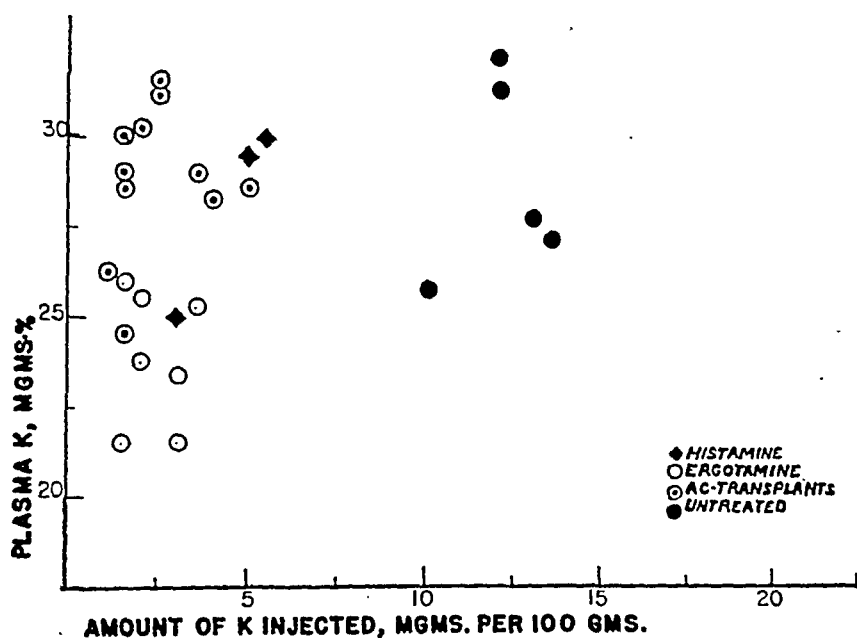


Fig. 1. Comparison of the plasma K values obtained at the time of circulatory collapse in rats after repeated injections of the standard dose of KCl by vein. Solid dot, untreated, intact rats. Solid diamond, histamine-treated rats. Open circle, ergotamine-treated rats. Dotted circle, untreated rats with adrenocortical grafts.

per cent (see fig. 1). Resting serum K levels under anesthesia were previously found to be 21.3 mgm. per cent for the untreated rats and the ET-rats and 25.6 mgm. per cent for ACT-rats (1, 5); thus we have a 39.9 per cent rise in the normal, and a 12.2 per cent and 11.1 per cent rise in the ET-rats and ACT-rats respectively.

3. *The reaction of rats, pre-treated with histamine to intravenous injections of KCl.* Rats treated with 10 mgm. histamine per 100 grams body weight showed responses to the initial injection of intravenous K which did not differ materially from the reaction of untreated, normal rats, despite a low, stable level of blood pressure (average 52 mm. Hg). The tolerated load on continued

injection of KCl, however, was less than one-half the normal (table 3, fig. 1). In the three histamine treated rats, after 6, 10 and 11 injections, the plasma values were 25.0, 29.5 and 30.4 mgm. per cent respectively.

The blood pressure in normal rats treated with ergotamine averaged 118 mm. Hg (60 per cent above the average initial value). Histamine lowered this precipitately to an average of 65.3 mm. Hg, at which level it was maintained essentially without change. With the first injection of KCl a fall in blood pres-

TABLE 3

*Comparison of the effect of histamine on the sensitivity of the cardiovascular response to intravenous injections of KCl in untreated rats and rats treated with ergotamine*

CLASS NO.	NORMAL RATS (3)		ET-RATS (5)	
	Average	Range	Average	Range
Initial mean pressure	70	65-82	67	50-80
Mean pressure after ergotamine			118	108-148
Mean pressure after histamine	52	47-60	65	48-72
Mean pressure before last KCl injection	54	48-68	65	48-72
Number of injections of KCl	9 $\pm 2.1$	6-11	1	Partial or full
Response to KCl on first injection	Fall insign.		Circulatory collapse	
Response to KCl on last injection, mm. Hg	-38	32-43		
Per cent fall	70	59-83		
Plasma K after last injection	28.16 $\pm 2.26$		Not obtainable	
Remarks	Recovery assisted		No recovery with assistance	

sure occurred followed by rapid circulatory failure. In all 4 cases circulatory failure was produced with one partial or one full dose of KCl.

Thus, the histamine-treated normal rats simulated in a way the cortical deficient ACT-rats as the changes in circulating K indicated, although the resistance of the former still was somewhat the better of the two. On the other hand, the histamine-treated ET-rat differed in its response to intravenous KCl. In this circumstance, the spectacular, increased toxicity of injected potassium was in no way comparable to that characteristic of the other groups.

This may indeed indicate the effect of extensive imbalance of autonomic activity in conjunction with an increased demand for adrenocortical protection (fig. 2).

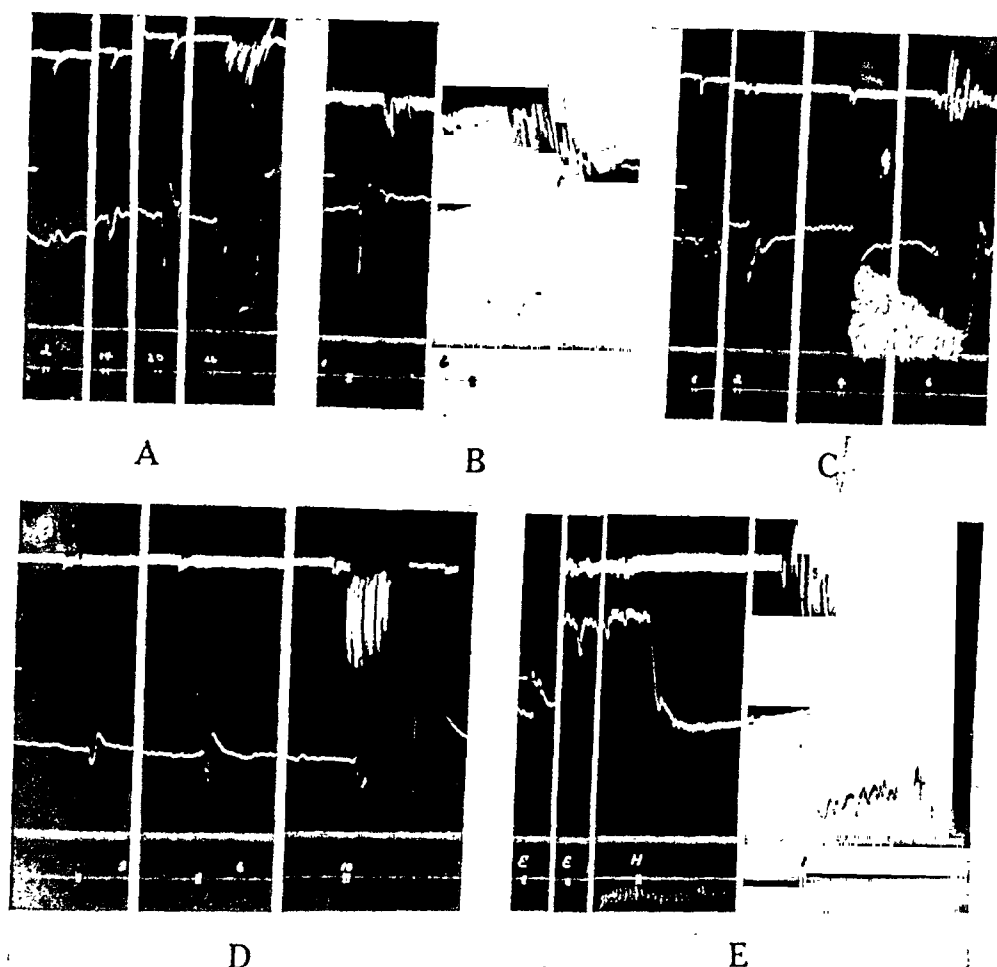


Fig. 2. The effect of repeated injections of 0.05 ml. 2 per cent KCl per 100 grams body weight by vein on the blood pressure of rats.

A. Untreated, intact rat. B. Untreated rat with adrenocortical transplants only. Effect of the 1st and 6th (last) injection of KCl. C. Intact rat treated with 0.5 mgm. ergotamine tartrate per 100 grams body weight. Effect of the 1st, 2nd, 4th and 6th injection of KCl. Ergotamine administered between the 1st and 2nd injections. D. Intact rat treated with 10 mgm. per 100 grams body weight of histamine. Effect of the 3rd, 6th and 10th (last) injection of KCl. E. Intact rat treated with ergotamine, 0.5 mgm. per 100 grams body weight.

At E, effect of 0.05 ml. of 1:200,000 epinephrine before and after ergotamine. At H, effect of 10 mgm. per 100 grams body weight of histamine. At I, effect of the 1st injection of KCl.

Upper curve, respiration. Lower curve, blood pressure, 100 mm. Hg indicated on left margin.

DISCUSSION. By maintaining the rate of intravenous administration of K strictly uniform in all groups of rats consistent differences were obtained. Cir-

culatory collapse was induced in the ergotamine-treated rat with less than one-third the amount required by the untreated rat and at lower plasma K values. This indicated that ergotamine sensitized the cardiovascular system to artificial rises in circulating potassium rather than influenced the effectiveness or mobilization of adrenocortical hormones.

The susceptibility of the ergotamine-treated rat superficially resembled that of the ACT-rat. In both groups the final depressor reactions occurred after the same small rise of 11 to 12 per cent in plasma K but in the ACT-rat the initial and terminal values were higher. This might be taken to signify that the inability to effect K-clearance, equal to that of the control rat, had a similar origin relative to the depression of adrenergic activity. In the ACT-rat the situation was identical as evidence was lacking of any significant degree of sympathetic disturbance other than reversal of the adrenalin pressor effect. On the other hand, the inability to shift water and electrolytes has been attributed to the functional inefficiency of grafted or regenerated cortical tissue (4, 12). In the light of Vogt's evidence that adrenalin prolongs the release of cortin (13) one might suspect that medullary lack or the effect of sympatholytic agents could reduce the efficiency of cortical activity. In any event, it seemed highly unlikely that the same degree of cortical deficiency and consequently the same degree of K susceptibility would obtain in both groups. Giroud (6) has found that regenerated cortex per gram is less potent than normal cortical tissue. It has also been shown that the histamine susceptibility of the ergotamine-treated rat is not comparable to that of the ACT-rat (1). The fact that the rat subjected to some degree of cortical depletion by histamine showed a similar increased sensitivity to K and that circulatory collapse occurred with plasma K values in the vicinity of those occurring in the normal and ACT-rat (all of which were higher than the ET-rat) indicated that there was no serious impairment of sympathetic function in the ACT-rat but rather an inadequate cortical protection. The acute sensitivity to K in the rat treated with ergotamine followed by a small amount of histamine seems to reflect something more than the additive effects of both deficiencies. With ergotamine tartrate, unlike other sympatholytic agents such as DHE-45, the contributions of other typical side-reactions require further investigation. Of these, the effect of ergotamine on the peripheral circulation to maintain a higher degree of resistance under all conditions of these experiments could well be a dynamic factor facilitating circulatory failure. Nevertheless, the experiments show that moderate deficiencies of adrenocortical activity favor a potassium-induced circulatory collapse by permitting a more rapid rise in extracellular K, whereas inactivation of adrenergic mechanisms by sympatholytic agents may sensitize the cardiovascular system of the rat to K at low values. The combination of both dysfunctions produced an acute susceptibility to potassium.

#### CONCLUSIONS

1. Ergotamine tartrate increased about three-fold the susceptibility of intact rats to repeated injections of KCl, as evidenced by circulatory collapse. Rats

with autoplasmic, adrenocortical transplants showed a comparable sensitivity of the cardiovascular system.

2. At the time of collapse, the plasma potassium had attained equally high levels in normal rats, histamine-treated rats and rats with transplanted adrenocortical tissue. The level, however, was definitely lower in rats subjected to ergotamine.

3. The increased susceptibility of ergotamine-treated rats to potassium was further enhanced by histamine, such that a single injection of KCl would produce prompt circulatory failure.

*Acknowledgment.* The author wishes to thank Drs. H. O. Haterius, A. H. Hegnauer and L. C. Wyman for their kindly interest in this study.

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# INDIVIDUAL DIFFERENCES IN RESPIRATORY GAS EXCHANGE DURING RECOVERY FROM MODERATE EXERCISE<sup>1</sup>

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The majority of studies on metabolic recovery from exercise have been related to strenuous, often exhausting exercise, the recovery from which may take several hours (Hill, Long, and Lupton, 1924; Margaria, Edwards, and Dill, 1933; Gemmill, 1931; and others). Hill et al. (1924) first pointed out that recovery curves for oxygen consumption are exponential, which has subsequently been confirmed by other investigators. (Simonson, 1926; Liebenow, 1928; Margaria, Edwards, and Dill, 1933.) According to Hill (1924) and Margaria et al. (1933), the oxygen recovery curve after strenuous exercise is composed of two exponential curves, an initial rapid component lasting a few minutes, and a longer component of several hours' duration depending upon the exercise intensity. After moderate exercise, the recovery curve is composed entirely of the first component. Simonson (1926) reported on the rapid recovery phase of three individuals after light exercise and noted that recovery curves of oxygen consumption were exponential with apparent individual differences of velocity constants. Hebestreit (1929) studied the relationships of the recovery processes after fairly vigorous exercise. Margaria et al. (1933) discussed the initial rapid recovery phase after strenuous exercise, but made a more detailed study of the slower recovery component.

The following report is concerned with an extensive study of recovery curves of both carbon dioxide elimination and oxygen consumption after moderate exercise of humans. For this purpose the thermal conductivity method of analysis has been adapted for continual and simultaneous measurements of carbon dioxide and oxygen in expired air permitting rapid changes of gas exchange to be followed during and after exercise.

APPARATUS FOR ANALYSES OF CARBON DIOXIDE AND OXYGEN. Analysis of gases by the thermal conductivity method has been used extensively in industrial fields; however, its application in physiological research has been reported by comparatively few investigators. Noyons (1922) described an apparatus for measuring CO<sub>2</sub> in samples of expired air. A portable instrument for clinical estimation of alveolar CO<sub>2</sub> has been employed by Hill (1922). Instruments for analyses of CO<sub>2</sub> and O<sub>2</sub> in alveolar air have been developed by Ledig and Lyman (1927), and Leeds and Northrup Company. Rabinowich and Bazin (1926) used a katharometer for continual measurements of CO<sub>2</sub> in expired air

<sup>1</sup> The work in this paper was initially done under a contract between the University of California and the Office of Scientific Research and Development, and later between the University of California and the Aeromedical Laboratory, Wright Field, Ohio.

and Rein (1933) employed the thermal conductivity method for continuous measurement of  $O_2$  in expired air.

The following is a brief description of the use of thermal conductivity analyzers for continual and simultaneous measurements of  $CO_2$  and  $O_2$  in expired air.

The principles and theory of the thermal conductivity method for analyses of gases have been reviewed by Palmer and Weaver (1924) and Daynes (1933). In brief, the method consists of a comparison of the resistance of two electrically heated platinum wires surrounded by a gas for analysis and a reference gas. If these gases have different thermal conductivities, the heat loss from the wires is altered with a corresponding change in the temperature and resistance of the wires. By suitable arrangement in a Wheatstone bridge circuit, the relative change of resistance of the wires affords a means of analysis of a mixture of gases after an empirical calibration of the apparatus.

Due to the similarity of the conductivity coefficients, measurements of oxygen in an oxygen-nitrogen mixture requires high sensitivity, yet the volume of the cell must be kept small in order to insure a rapid flushing out time. The desired sensitivity for the oxygen analyzer was obtained by increasing the length of the platinum wire while the volume of the cell was kept at a minimum by doubling the wire. The increased sensitivity obtained by this construction permitted the cell to be operated at a lower current with a corresponding increase in stability of the zero readings.

Analyzing and reference cells for carbon dioxide and oxygen were constructed of equal volumes from glass tubing with the platinum wires, held taut with helical copper coils, sealed into place with cement.

The cells were constructed in pairs, one for the reference gas and one for analysis, mounted on bakelite bases and immersed in an oil bath maintained at a constant temperature in a larger water bath.

The circuits used were of the parallel bridge type (see Daynes, 1933) with manganin arm resistances and galvanometers with sensitivities of 0.02 micro-ampere per division. The current flow from two storage batteries through the platinum wires was controlled with a voltmeter sensitive to a change of 0.01 volt. Resistance changes of the platinum wires, reflecting changes of gas composition in the analyzing cells, were measured by the null method, the circuits being balanced by manual operation of the slide wires.

Empirical calibration of the instrument was made with known mixtures of gases previously obtained by compressing expired air into steel cylinders.<sup>2</sup> Equations<sup>3</sup> derived from the calibration curves are used to transform slide

<sup>2</sup> Mixtures of commercial oxygen, nitrogen, and carbon dioxide were found to give erroneous calibration curves which is believed to be due to different argon-nitrogen ratios in the mixtures as compared to air.

<sup>3</sup> These equations are per cent  $CO_2 = K_1 D_{CO_2} - K_2 D_{O_2} + K_3$ , and per cent  $O_2$  (diff. from air) =  $K_4 D_{O_2}$  (1 - per cent  $CO_2/100$ ).  $D_{O_2}$  and  $D_{CO_2}$  are slide wire readings for carbon dioxide and oxygen respectively and  $K_1$ ,  $K_2$ ,  $K_3$ , and  $K_4$  are constants derived from the calibration curves;  $K_4$  includes a correction for the difference in volume of inspired and expired air.

wire readings directly to percentage gases with an accuracy of  $\pm 0.03$  per cent  $\text{CO}_2$  and  $\text{O}_2$ .

Arrangement of the apparatus for analyses of expired air is illustrated in figure 1. One way valves in the mask and connecting tubes direct the expired air through a cylinder with baffles into a large gasometer. The cylinder mixes the expired air resulting in a more uniform composition for analysis. A small continuous flow of expired air is drawn through each of the three-way capillary stopcocks inserted into the T-tube placed after the cylinder. One stream of air is drawn through a small  $\text{CaCl}_2$  tube, to remove water vapor, and into the carbon dioxide analyzing cell; the other stream is drawn through a suitable water vapor and carbon dioxide absorbent and into the oxygen analyzing cell.

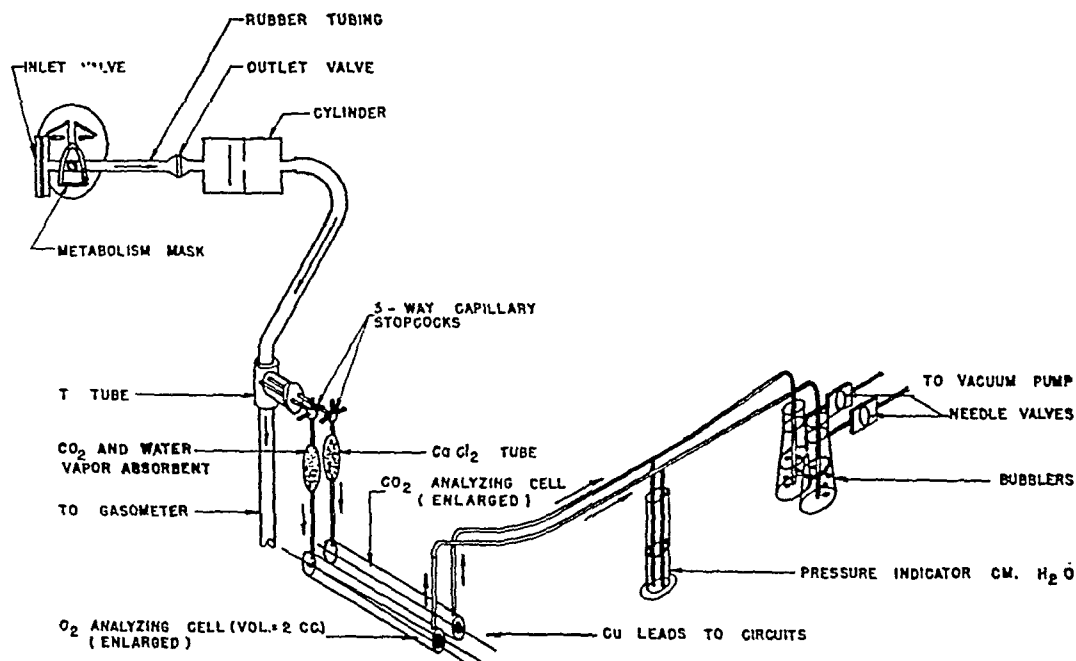


Fig. 1. Arrangement of apparatus for measuring oxygen consumption and carbon dioxide elimination.

Expired air for experimental readings or atmospheric air for checking zero readings may be drawn into the cells according to the positions of the three-way stopcocks, the rate of flow being accurately controlled by needle valves and bubblers. Constrictions in the capillary tubes lower the pressure in the cells by 6 to 10 cm. of water, thus preventing rhythmical fluctuations of pressure, due to the subject's breathing, from being transmitted to the cells.

Actual analyses of expired air represent the average percentages of the gases over a time interval depending upon the volume of the cells and the rate of flow of gas through them. These factors were adjusted so that each measurement of carbon dioxide and oxygen represents an average value for an interval of twenty seconds. In certain experiments this has been reduced to fifteen

seconds; however, it is not feasible to balance the circuits manually in less time than this. Moreover, for metabolic experiments there is no necessity for shorter periods than twenty seconds as variations in ventilation determinations become excessively large.

Ventilation rates are measured in a 100 liter capacity gasometer. With high ventilation rates, measurements are made every twenty seconds corresponding with the analyses of carbon dioxide and oxygen. With low ventilation rates, as during resting metabolism, the volume determinations are made every minute.

A generalized procedure for measuring metabolism during or after exercise is as follows: the subject rests in a comfortable chair, the metabolism mask is strapped on and an exercise initiated. The metabolism may be measured

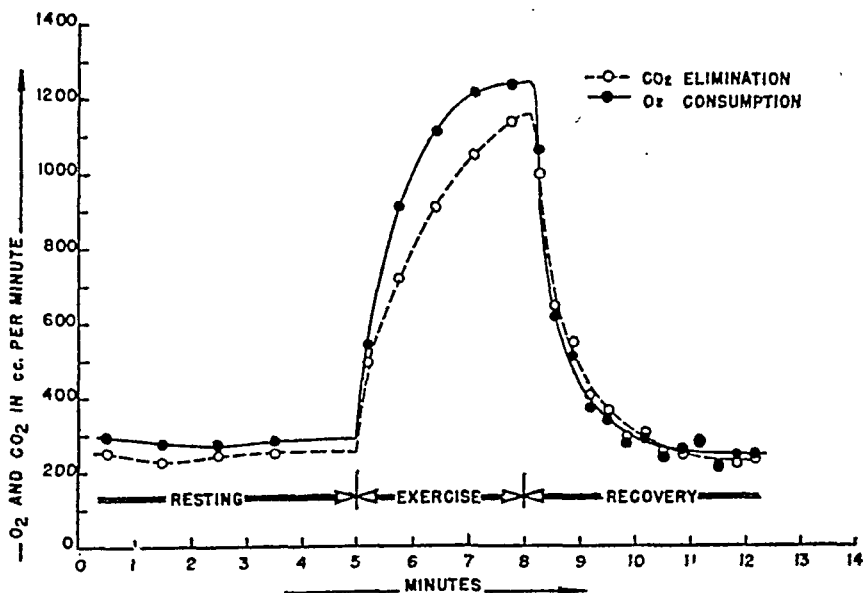


Fig. 2. O<sub>2</sub> Consumption and CO<sub>2</sub> elimination during and after the standard step-up exercise. (W. E. B., 8/27/46)

at any time during or after the exercise by drawing the expired air through the analyzing cells and measuring ventilation rates. Slide wire readings are converted by means of equations to percentages of gases and, with corresponding minute ventilation measurements, the gas exchange for 20 second intervals is obtained.

Figure 2 illustrates measurements, by this method, of carbon dioxide elimination and oxygen consumption of a subject during and after a three minute step-up exercise.

After moderate exercise of the type used in the following studies, the metabolism reaches pre-exercise resting levels 6 to 10 minutes after completing the exercise; therefore resting metabolism may be measured either before exercise or after the recovery period.

*Respiratory gas exchange during recovery from exercise.* Initial experiments were carried out to analyze the gas exchange curves during and after moderate exercise, for the purpose of measuring individual differences.

As originally noted by Hill (1924), the recovery curves for  $O_2$  consumption after moderate exercise are logarithmic, following the equation  $A = A_0 e^{-kt}$ . This equation is equally applicable to recovery curves for  $CO_2$  elimination where  $A$  is the rate of  $CO_2$  elimination at time  $t$ ,  $A_0$  is the rate at zero time, and  $k$  is the velocity constant. The two factors that determine these recovery curves, i.e., rate of ventilation and percentage of gases, are also logarithmic; however, fluctuations from the theoretical curves may be considerable. It was also noted that in general the curves for  $CO_2$  elimination and  $O_2$  consumption as they rise from the resting values and approach the steady state levels are logarithmic, using the steady state values as base lines. However, individual comparisons of velocity constants of these curves were difficult due to erratic results, presumably caused by uneven ventilation and circulation during exercise. Recovery rate measurements were found to be more practical for large scale comparisons between individuals, the most suitable of these exercises being step-up or stationary bicycle exercises. The recovery phases of these exercises, as compared to arm or knee bend exercises, are more extended and are not complicated by post exercise rises of metabolism.<sup>4</sup> Simple step-up exercises were selected as being easier for aged or feeble subjects to perform than bicycle exercises.

In order to make an extensive study of the recovery phase, a standard leg exercise was chosen which consists of 20 steps/minute onto a 9-inch platform for three minutes.<sup>5</sup> This duration permits 95-98 per cent attainment of steady state values with  $O_2$  consumption levels ranging from 800-1100 cc.  $O_2$ /minute above resting. With this intensity of exercise there is no detectable slow recovery component and pre-exercise resting levels are reached 6 to 10 minutes after the exercise.

Recovery rates are determined by plotting  $CO_2$  elimination and  $O_2$  consumption, after subtraction of resting values, semi-logarithmically against time, as illustrated in figure 3. The slopes of the straight lines represent recovery rates and are expressed as half-time constants, i.e., the time when the rate of gas exchanged is one-half the amount at the onset of recovery. Thus slow or fast recovery rates are represented by large or small half-time constants respectively. Integration of the curves represents the  $O_2$  debt and excess  $CO_2$  during the recovery period. The S-shape deviations from the exponential function

<sup>4</sup> Immediately after an isotonic bar lifting arm exercise the metabolism rises above the exercise levels and then exponentially approaches the resting values. This post-exercise rise indicates there is restricted circulation during the exercise as a result of the continually contracting muscles. If the arms are periodically relaxed during the exercise, the exercise metabolic levels increase and the post-exercise rise disappears. This phenomenon may be observed in the recovery phase of leg exercises depending upon whether or not the leg muscles are periodically relaxed during the exercise.

<sup>5</sup> In cadence with a metronome the subject performs the following four cycle exercise: left foot up, right foot up, left foot down, right foot down.

as described by Hebestreit (1929) have not been observed in these experiments.

Ventilation rate measurements and gas analyses are sources of experimental error. These errors are considered to be very slight as the percentage of gases may be analyzed to  $\pm 0.03$  per cent and the volume of expired air measured to  $\pm 0.05$  liter. Although there may be fluctuations in ventilation rates and percentage gases over short time intervals due to intermittent flowing of expired air, these are compensating errors and tend to be smoothed out in the final recovery curve. Determination of the base lines which the recovery curves approach, i.e., the resting metabolism, and graphic measurements of the recovery curve slopes are other sources of errors. Variations of resting metabolism may amount

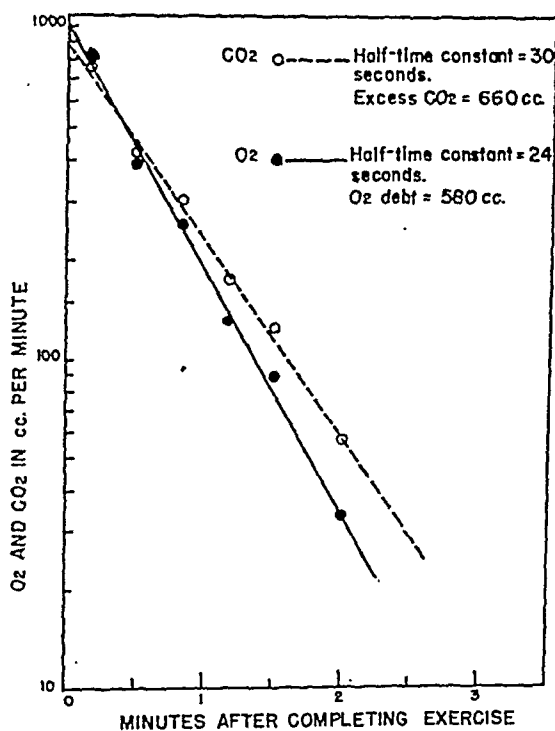


Fig. 3. Semi-logarithmic plot of CO<sub>2</sub> elimination and O<sub>2</sub> consumption (above resting) during recovery from a step-up exercise (data on W. B. from fig. 2).

to as much as  $\pm 10$  per cent, which, however, are reflected by only  $\pm 2-3$  per cent errors in the recovery constants. Graphic determination of the recovery curve slopes is probably the largest source of error since the recovery curves are seldom perfectly logarithmic and subjective errors are estimated to be from 1-10 per cent of the recovery constants.

*Reliability and variability of recovery measurements.* Preliminary results indicated possible differences in recovery rates between individuals in addition to variation within the individuals. In order to establish the magnitude of these variations, as well as the reliability of the measurements, tests and retests were carried out on a number of subjects.

The previously described step-up exercise was used as a standard exercise

for the test and retest. Male subjects, with ages ranging from 18-68 years, included the laboratory staff, students, janitors, and office workers. Tests and retests, after previous instructions and a practice test, were usually carried out at the same time on consecutive days. The recovery curves were analyzed for the  $O_2$  and  $CO_2$  half-time recovery constants,  $O_2$  debt, and total excess  $CO_2$ . The results are summarized in table 1. Oxygen debts and excess carbon dioxide were determined by integration of the recovery curves with corrections for body weights of the individuals. The reliabilities of these measurements were determined by test-retest correlations.

The conclusions that may be drawn regarding the reliability and variability of the recovery measurements are as follows:

1. A high reliability of the  $CO_2$  time constants ( $r = 0.74$ ) indicates that

TABLE 1

*Reliability of measurements of half-time recovery constants,  $O_2$  debt and excess  $CO_2$  after a standard exercise test*

MEASUREMENTS	NUMBER OF SUBJECTS		MEAN	STANDARD DEVIATION	RANGE	RELIABILITY ( $r$ )
Time constants in seconds of $CO_2$ elimination during recovery	38	Test	45.5	10.7	30-68	0.74
		Retest	44.2	10.9	30-73	
Excess $CO_2$ eliminated during recovery, cc. $CO_2$ /kgm. body wt.	29	Test	14.2	3.9	8.5-25.4	0.87
		Retest	14.3	3.9	9.2-21.9	
Time constants in seconds of $O_2$ consumption during recovery	36	Test	32.0	6.8	20-46	0.55
		Retest	30.6	7.2	17-54	
$O_2$ debt, cc. $O_2$ /kgm. body wt.	28	Test	11.75	2.7	7.1-19.8	0.67
		Retest	11.73	3.2	6.4-17.7	

there are definite interindividual differences as regards this measurement. The standard error of the measurement ( $\sigma_m$ ) is  $\pm 5.5$  seconds or  $\pm 12$  per cent of the mean.

2. The reliability of the  $O_2$  time constants is considerably lower ( $r = 0.55$ ) indicating either lower interindividual and/or higher intraindividual differences. The standard error of the measurement is  $\pm 4.7$  seconds or  $\pm 15$  per cent of the mean.

3. The average of the  $CO_2$  time constants of 36 subjects (43.9 sec.) is 40 per cent more than the average of the  $O_2$  time constants (31.3 sec.) on the same individuals. Thus the  $O_2$  debt is paid at a considerably more rapid rate than the corresponding excess  $CO_2$  is eliminated. This difference, as will be seen later, depends upon the age of the individual.

4. Although the  $CO_2$  time constants are longer than the  $O_2$  constants, these

two measurements are not independently variable as the correlation ( $r$ ) between them is 0.84.

5. There is no significant correlation between body weights of subjects and their respective recovery constants.

6. Calculations of  $O_2$  debt and excess  $CO_2$ , obtained by integration of the recovery curves, are directly proportional to the time constants and the values of gas exchange at zero recovery time. Their reliabilities are slightly higher than the corresponding recovery constants (table 1), probably due to a low, although significant, negative correlation between the time constants and gas exchange at the onset of recovery. Thus a slow or fast recovery rate is correlated with a respectively low or high initial recovery metabolism; this inverse correlation consequently minimizes variations of the  $O_2$  debt and excess  $CO_2$ . However, little advantage is gained by their calculation and in subsequent data only recovery constants are listed.

7. In general the experimental errors are less than the recovery rate variations of any one individual. Accordingly these variations are predominately

TABLE 2

*Comparisons of average deviations of the tests from the retests fifteen minutes and one week apart*

Means of the  $CO_2$  and  $O_2$  recovery constants are 38.9 and 28.2 seconds respectively.

TIME INTERVAL BETWEEN TEST AND RETEST	NUMBER OF SUBJECTS	AVERAGE DEVIATION OF $CO_2$ RECOVERY CONSTANTS	AVERAGE DEVIATION OF $O_2$ RECOVERY CONSTANTS
		<i>seconds</i>	<i>seconds</i>
Fifteen minutes.....	27	2.76	4.13
One week.....	27	3.09	4.60

due to intrinsic factors in the individual and are only slightly affected by variations in the time interval between tests. For example, the average deviation between tests and retests fifteen minutes apart is 90 per cent as great as the average deviation between tests one to two weeks apart on the same subjects (table 2).

*Correlation of age with recovery rates.* Since a heterogeneous age group was used for the reliability measurements, it is possible from the data to establish the relation between age and recovery rates. The test and retest time constants for each individual were averaged and correlated with the age of the person. Included are time constants of a few older, weaker subjects who performed a milder exercise than the standard exercise, a procedure which decreases the time constants slightly. The results, illustrated in figure 4, show that the  $CO_2$  time constants are higher with increasing age of the subject, the correlation being 0.75. The effect of age on the  $O_2$  time constants is not as great ( $r = 0.43$ ), differences appearing mainly between the youngest and oldest subjects (fig. 4). This lower correlation may be due to less reliability of the  $O_2$  time constants or to a true differential influence of age on the recovery rates. If possible dif-

ferences in reliabilities of the  $O_2$  and  $CO_2$  recovery constants are eliminated by correction for attenuation in the criterion measurement (Guilford, J. P., 1942), the resulting correlations of age with  $CO_2$  and  $O_2$  recovery rates are 0.93 and 0.71. This establishes an actual differential effect of age on the recovery constants and it is concluded that with increasing age there is considerable slowing of  $CO_2$  elimination during recovery, whereas the rate of payment of  $O_2$  debts is not as greatly changed.

*Time of day and recovery constants.* The time of day apparently has little or no effect on the recovery constants as there was no significant difference between the average recovery constants of 20 subjects tested during the morning

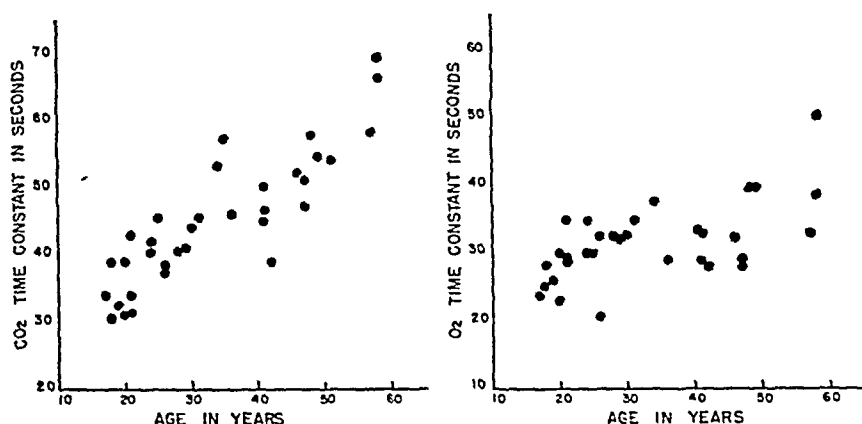


Fig. 4. Effect of age on recovery rates of  $CO_2$  elimination and  $O_2$  consumption after a standard step-up exercise. Each point is the average of two or more tests on the individual.

TABLE 3

*Time of day and recovery constants*

TIME	NUMBER OF SUBJECTS	AVERAGE OF $CO_2$ RECOVERY CONSTANTS	AVERAGE OF $O_2$ RECOVERY CONSTANTS
		<i>seconds</i>	<i>seconds</i>
9 a.m.-12 m.....	20	$36.7 \pm 3.2$	$27.5 \pm 3.7$
1 p.m.- 4 p.m.....	22	$40.0 \pm 2.3$	$28.5 \pm 2.1$

as compared to 22 subjects of comparable age tested in the afternoon (4 tests on each individual, table 3).

There may be a difference if the exercise is initiated from basal conditions, as recovery constants of a subject under near basal conditions were significantly greater (10 per cent) than the corresponding afternoon recovery constants.

*Effect of rate of work and duration of exercise on recovery constants.* For exercise tests on aged subjects or on subjects with pathological conditions, the standard exercise is too strenuous and must be lowered in accordance with the physical condition of the individual. This was accomplished by a reduction of the intensity and/or the duration of the standard exercise. It was thus necessary to know the effect of these modifications on the recovery rates.

For investigating the effect of work rate, two widely different levels were chosen, a rate of 12.5 steps/minute as compared to a more vigorous exercise of 25 steps/minute on a 9 inch platform. Average oxygen consumption levels above resting were 790 cc./minute and 1400 cc./minute respectively. The recovery constants of twelve subjects exercised at these two levels (table 4) show that a 50 per cent reduction of exercise intensity results in a slight, although significant, decrease (12 per cent) in the  $\text{CO}_2$  time constants,<sup>6</sup> whereas the  $\text{O}_2$  time constants are not significantly changed. Accordingly it may be assumed that exercise rates deviating moderately from the standard exercise are reflected by only slight changes in the recovery constants; and although corrections may be made, the changes are small compared to the individual variations.

The effect of exercise duration on the recovery constants was measured by comparing recoveries from one minute and three minute exercises, both at a rate of 20 steps per minute. After one minute of exercise at this rate, the metabolic

TABLE 4  
*Effects of intensity and duration of exercise on half-time constants of recovery*

VARIABLES	NUMBER OF SUBJECTS	EXERCISE CONDITIONS	METABOLISM	AVERAGE OF HALF-TIME CONSTANTS OF $\text{CO}_2$ ELIMINATION	AVERAGE OF HALF-TIME CONSTANTS OF $\text{O}_2$ CONSUMPTION
				<i>seconds</i>	<i>seconds</i>
Rate of work	12	12½ steps/min. for 3 min.	790 cc. $\text{O}_2$ /min., average	$38.2 \pm 4.6$	$29.7 \pm 4.6$
	12	25 steps/min. for 3 min.	1400 cc. $\text{O}_2$ /min., average	$43.5 \pm 5.5$	$30.3 \pm 2.2$
Duration of exercise	9	20 steps/min. for 1 min.	70-80% of steady state values	$38.8 \pm 3.3$	$31.1 \pm 2.3$
	9	20 steps/min. for 3 min.	95-98% of steady state values	$41.6 \pm 3.8$	$31.3 \pm 2.8$

levels of most subjects are 70-80 per cent of steady state values as compared to approximately 95 per cent in three minutes. The average recovery constants corresponding to these different exercise durations are not significantly different, showing that comparisons of recovery constants of subjects who have not attained steady states are feasible (table 4). The advantage, however, of a longer exercise is that the recovery curves begin at a higher level and consequently are lengthened permitting more accurate measurements of the recovery slopes. Recovery from exercises longer than three minutes was measured on a few subjects with no apparent change in recovery constants as compared to those above.

*Physical training and recovery constants.* The subjects in various age groups (from fig. 4) were classified as being in good, medium, or poor physical condition

<sup>6</sup> The standard exercise (20 steps/min.)  $\text{CO}_2$  recovery constants of a few of these subjects were found to fall between these two values; while very strenuous exercise increased the time constants by almost one-half. The relationship may be linear. Oxygen recovery constants, after nearly maximal work rates, also increased.

and an attempt was made to correlate condition with recovery constants. The data are rather limited, but indicate that in any age group the more physically fit individuals tend to have smaller recovery constants. The following data lend support to the above conclusion.

In November of 1945 the recovery constants were accurately determined of a laboratory staff member in moderate physical condition. Six months later it was noticed that this subject's recovery constants were significantly lower (table 5); this is ascribed to a considerably improved physical condition, the subject having undergone a rigorous training comparable to that of college athletes. The recovery constants of other staff members showed no particular change during this time. This is in agreement with the observation of Simonson (1926) that training apparently increases the recovery rates from moderate exercise.

TABLE 5

*Effect of physical fitness on the half-time recovery constants of subject E.M.*

CONDITION OF SUBJECT	NUMBER OF TESTS	TIME CONSTANTS IN SECONDS OF CO <sub>2</sub> ELIMINATION	TIME CONSTANTS IN SECONDS OF O <sub>2</sub> CONSUMPTION
Before training, Oct.-Nov., 1945.....	12	35.0 $\pm$ 2.5	30.8 $\pm$ 2.0
After training, June-July, 1946.....	13	29.2 $\pm$ 2.0	26.0 $\pm$ 2.4
Decrease after training.....		17% $t = 4.11$ (Significant at 1% level)	16% $t = 3.68$ (Significant at 1% level)

DISCUSSION. The reliabilities of the recovery constants (table 1) were determined for a heterogeneous age group. Since age differentially influences the magnitudes of the CO<sub>2</sub> and O<sub>2</sub> recovery constants, the respective reliabilities are likewise affected. If the age factor is eliminated by the use of a first order partial correlation (Guilford, J. P., 1942), the reliabilities become 0.21 for both the O<sub>2</sub> and CO<sub>2</sub> recovery constants.<sup>7</sup>

Segregation of the undoubtedly complex factors that cause day to day variations of recovery constants in an individual has not been attempted. Such factors may be related to conditions of the circulatory system. Interindividual differences, aside from age, are probably due in part to physical training as there is the indication that in any age group the more physically active individuals

<sup>7</sup> Following the completion of this work data have become available for determining reliabilities of these measurements on a nearly homogeneous age group of 38 subjects 17-23 years of age. Two immediately consecutive exercise tests were given one day followed by two tests one week later. Reliabilities of the CO<sub>2</sub> and O<sub>2</sub> recovery constants using the initial measurements on each day as tests and retests, are 0.41 and 0.37 respectively. If the second measurements are used for tests and retests, the reliabilities are 0.67 and 0.37. A preliminary exercise evidently lowers the intra-individual variation of the CO<sub>2</sub> recovery constants. These reliabilities are greater for this age group than theoretically predicted.

tend to have lower recovery time constants. The influence of age itself might be considered as due to poorer physical condition resulting from decreased physical activity with increasing age; however, there may be a true age effect since the physically vigorous older individuals had slower recovery rates than less active younger subjects.

The limiting factors in the rate of  $O_2$  consumption and  $CO_2$  elimination after moderate exercise are not definitely known although there is evidence that they may be circulatory in nature. Jones (1946) has demonstrated that exchange of inert gases between tissues and blood and elimination through the lungs is limited by circulation of the blood and solubility of the gases. Exchange of  $CO_2$  and  $O_2$  is complicated by chemical reactions in the blood and tissues; however there is evidence that these reactions are extremely rapid (Millikan, 1936; Roughton, 1935). The efficiency of lung ventilation is not considered as a limiting factor as there is no correlation between ventilation efficiency, as measured by  $N_2$  washing out rates from the lungs while breathing pure  $O_2$ , and the recovery constants (unpublished data).

The concept of circulatory limiting factors is supported by recovery rate measurements after a single leg exercise with a pressure cuff placed around the thigh. Although under these conditions the recovery curves are still exponential, the recovery constants of the leg may be increased as much as 50 percent depending upon the tightness of the cuff. Also, increases of circulation, as measured by the inert gas exchange technique (Jones, 1946), are not linearly proportional to increases in exercise intensity, thus conforming to the gradual lengthening of the recovery constants with increasing exercise intensity.

Finally, recovery rates of  $O_2$  consumption of subjects with circulatory insufficiencies are delayed (see review by Simonson and Enzer, 1942) which suggests circulation as being an important limiting factor.

In accordance with the above views, factors other than actual blood flow that might influence the recovery rates are  $O_2$  utilization coefficients and the  $CO_2$  carrying capacity or unloading capacity at the lungs. The difference between the  $O_2$  and  $CO_2$  recovery constants would then depend upon these factors. For example, subjects with polycythemia vera have significantly slower than normal  $CO_2$  recovery rates; whereas, despite a slower circulation (Gibson, Harris, and Swigert, 1939), the  $O_2$  recovery rates are normal, presumably because the  $O_2$  carrying capacity of the blood is greatly increased above normal (to be published).

These exercise recovery measurements have been developed mainly as a study of normal exercise recovery; however, possible applications to pathological conditions have also been considered. Attention has been directed towards accumulating sufficient knowledge and experience with normal subjects, such as the recovery rates for various age groups, in order that deviations from the normal may be more accurately studied. Future investigations with these methods on subjects with pathological conditions of the circulatory system may prove of clinical value, and also may clarify the actual nature of the limiting factors.

## SUMMARY

1. The thermal conductivity method of gas analysis adapted for continual and simultaneous analyses of  $O_2$  and  $CO_2$  in expired air is described. The apparatus, with an accuracy of  $\pm 0.03$  per cent  $CO_2$  and  $O_2$ , is designed for following  $O_2$  consumption and  $CO_2$  elimination of humans during and after exercise.

2. Metabolic gas exchange curves during and after various types of moderate exercises were studied for individual differences. The recovery phase from simple step-up exercises was found to be practical for this purpose. The  $O_2$  consumption and  $CO_2$  elimination recovery curves are exponential, the half-time constants of which are functions of recovery rates; thus, a slow or fast recovery from exercise is represented by a large or small half-time constant respectively.

3. Reliability and variability of the time constants of recovery from a mild step-up exercise were determined by tests and retests on 38 male subjects of various ages. The reliabilities of the  $CO_2$  and  $O_2$  time constants are 0.74 and 0.55 respectively, with standard errors of measurements being  $\pm 12$  per cent and  $\pm 15$  per cent of the means.

4. The recovery rate variations of a subject are considered to be due mainly to intrinsic factors in the individual and are only slightly increased by lengthening the time interval between tests.

5. The average of the  $CO_2$  half-time recovery constants (43.9 sec.) of 36 subjects is 40 per cent more than the average  $O_2$  time constants (31.3 sec.); thus the rate of payment of the  $O_2$  debt is more rapid than elimination of the corresponding excess  $CO_2$ . The  $O_2$  and  $CO_2$  recovery constants, however, are not independently variable as the correlation between them is 0.84.

6. With increasing age of subjects there is increased slowness of  $CO_2$  elimination during recovery; the  $CO_2$  half-time recovery constants of 60 year old subjects are nearly double those of 20 year old subjects. The  $O_2$  recovery constants are apparently less affected by age, differences appearing mainly between the very youngest and oldest subjects. The age effect accounts to a large extent for the inter-individual differences in recovery rates. Elimination of the influence of age by a first order partial correlation results in a theoretical reliability of 0.21 for both  $CO_2$  and  $O_2$  recovery constants of a homogeneous age group.

7. Different intensities and durations of moderate exercise have little or no effect on the recovery constants which permits comparison of recovery rates between individuals who perform varying degrees of work.

8. In any age group the more physically fit individuals tend to have lower recovery constants, and in addition rigorous training of one subject brought about a 16 per cent reduction in the  $CO_2$  and  $O_2$  recovery constants.

9. The possibility is considered that the limiting factors in the rate of  $O_2$  consumption and  $CO_2$  elimination during recovery are circulatory in nature.

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# THE RELATION BETWEEN THE ESTERASE ACTIVITY OF THE BLOOD PLASMA AND OF THE LIVER OF THE DOG<sup>1</sup>

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A relation between liver injury and abnormal levels of plasma cholinesterase has been suspected in man on the basis of clinical statistics (1), and has been demonstrated in the laboratory in the rat (2). The nature of this relation, however, cannot be determined from the available data, and many of the points in question cannot be settled without the use of one of the larger laboratory animals. Since the known properties of the acetylcholine-hydrolyzing enzymes of human and of rat plasma resemble those of dog plasma cholinesterase (3), the dog seems suitable for this purpose. The present report is concerned with some of the factors involved in the maintenance of a normal plasma cholinesterase activity in the dog.

**GENERAL METHODS.** Cholinesterase activities were determined by the method of Ammon (4) using 0.08 M acetyl choline bromide<sup>2</sup> (once recrystallized) in 0.025 M sodium bicarbonate buffer pH 7.4—0.25 M NaCl, and are expressed as millimoles of acetyl choline hydrolyzed per hour by one liter of plasma or one kilogram of tissue. Results were checked repeatedly against a medium containing KCl, MgCl<sub>2</sub>, and CaCl<sub>2</sub> in addition to NaCl, but no significant differences between the two electrolyte media could be observed.<sup>3</sup> Blood was in all cases drawn from the radial vein and heparinized. Analyses for liver cholinesterase activities were performed by the same method, using fresh samples of 0.3 to 0.5 gram weight pressed gently between filter paper to remove excess blood, and homogenized with ten volumes of saline in the apparatus described by Potter and Elvehjem (5). The degree of dilution of the tissue was calculated directly from the fresh weight of tissue and the volume of saline used. In normal dogs the distribution of the cholinesterase activity throughout the liver appears to be approximately uniform (cf. table 1) so that satisfactory results can be obtained by the analysis of a single piece of tissue, such as can be obtained as a biopsy specimen. In dogs with liver disease the uniform distribution of the enzyme may be disturbed; to secure a representative sample of liver tissue for analysis, samples were taken from each lobe and combined into one. Storage of samples of intact liver in closed containers in the refrigerator led to losses of only a few percent of the initial cholinesterase activity over a period of one week.

<sup>1</sup> This work has been supported by grants from the U. S. Public Health Service, and the Ella Sachs Plotz Foundation.

<sup>2</sup> Kindly furnished by Hoffmann LaRoche, Inc.

<sup>3</sup> This observation has been repeated with purified human esterase preparations of very low ash content supplied through the kindness of Dr. E. J. Cohn of the Department of Physical Chemistry, Harvard Medical School.

Serum alkaline phosphatase activities were determined by the method of Shinowara, Jones and Reinhardt (6). Total plasma protein determinations were performed by the copper sulfate specific gravity method of Phillips *et al.* (7). Erythrocyte counts and hematocrits were determined by the conventional methods.

A special procedure was adopted for the determination of bromsulfalein (BSP) elimination. Seventy-five milligrams of the dye<sup>4</sup> in 5 percent solution were injected into a radial vein. Three venous blood samples were obtained, one prior to the injection, one three to five minutes after the injection, and a third five to ten minutes later. The magnitude of the interval between the second and third samples was immaterial, so long as its duration was known

TABLE 1

*The cholinesterase activity of samples of dog's liver taken from different lobes (mM/Kgm/hr.)*

DOG NO.				
70	660	665	715	650
71	1530	1485	1420	1320
75	1630	1540	1510	1430
77	2080	2000	1950	1870
69	2030	1880	1840	1780

TABLE 2

*The cholinesterase activity of the blood plasma of male and of female dogs*

	MALE	FEMALE
Number of animals.....	53	29
Mean cholinesterase activity.....	101.1	105.2 mM/L/hour
Standard deviations of single determinations.....	±28.1	±23.7
of the mean.....	±3.9	±4.4

$$t = 0.69, n = 82, P = 0.5$$

to within  $\pm 10$  seconds. The plasma was deproteinized by the addition of 5.0 cc. of acetone to 1.0 cc. of plasma, followed by centrifugation. Five cubic centimeters of the supernatant were transferred to a colorimeter tube, and the BSP color developed by the addition of two drops of 10 percent KOH. If necessary the solutions were cleared on the centrifuge, and the resulting color densities read in a Klett-Summerson photoelectric colorimeter, using the No. 54 (green) filter. In general the readings for the acetone-saline blank and for the control samples were identical. From the results the percent of dye eliminated in 5 minutes could be calculated using Bradley and Inglefinger's finding of an exponential time course of the decay of plasma BSP following a single injection of the dye in man (8) and in dog (9). The following formula was applied:

$$E = 100 \times \left( 1 - \frac{B_3^{5/I_{2,3}}}{B_2} \right)$$

where  $E = \% \text{ BSP}_{5 \text{ min.}}$ ,  $B_2$  and  $B_3 =$  concentration of BSP in second and in third samples, and  $I_{2,3} =$  interval in minutes between drawing of second and of third sample. This procedure obviates difficulties due to hemolysis, or to turbidity of the plasma samples, as well as to differences of plasma volumes

<sup>4</sup> The authors wish to thank Hynson, Westcott and Dunning, Inc. for supplying the Bromsulphalein used in these experiments.

among various dogs, and in the same dog during the course of an experiment. *BSP* recovery in this procedure is better than 95 percent except in samples showing an excessive degree of hemolysis never encountered during actual experiments. The average value of the % *BSP*<sub>5 min.</sub> in normal dogs was found to be 64 percent; even a small degree of impairment could be detected.

Determinations of plasma volume were carried out using an adaptation of the method described by Gibson and Evelyn (10) to the Klett-Summerson photoelectric colorimeter. Plasma volume studies were attempted in dogs with carbon tetrachloride poisoning but had to be abandoned because of the effect of the liver injury upon the apparent plasma volume: the administration of carbon tetrachloride was followed in each case by an increase of from 30 to 50 percent in the plasma volume as determined by the use of T 1824. Until this phenomenon has been investigated in some detail there seems to be little justification in reporting Evans' blue plasma volumes in this condition.

**RESULTS.** A. *The normal plasma cholinesterase activity of the dog, and its relation to sex, liver cholinesterase activity, and total plasma protein concentration.* Blood plasma and liver samples obtained from healthy dogs weighing between 8 and 25 kgm. were analyzed for their cholinesterase content. The dogs probably represent a fair sample of a population of healthy mongrel dogs. The nutritional status of this group, however, could not be controlled, and lack of uniformity in this regard may account for some of the scattering described below.

Table 2 summarizes data concerning the plasma cholinesterase activity of male as compared to female dogs. The mean values of the two series do not differ significantly, as shown by the t-test (11). Despite the small standard deviations of the means the standard deviations of single determinations are large, reflecting considerable variability in the normal plasma cholinesterase activity among dogs. Indeed, the normal range is so wide that it is not possible to detect abnormalities in the plasma cholinesterase activity of one animal by comparison with a series of normal values. Significant information can, however, be obtained if serial analyses can be carried out on an individual animal since the plasma cholinesterase activity of a healthy, well fed dog remains relatively constant over long periods of time.

Simultaneous determinations of liver and blood plasma cholinesterase activities revealed a significant degree of correlation (11) between these two variates (fig. 1).

One gram of liver contains about twelve times the cholinesterase activity of 1 cc. of blood plasma over the range of plasma cholinesterase values seen in this series.

Table 3 shows data collected for relatively few dogs, and indicates that a relation may also exist between the total liver cholinesterase activities and the plasma cholinesterase activity.

No significant degree of correlation could be detected between the total plasma protein concentration and the plasma cholinesterase activities observed in various dogs (44 dogs,  $r = 0.13$ ,  $P > 0.1$ ).

B. *The effect of toxic liver injury upon the plasma cholinesterase activity of healthy dogs.* Mongrel dogs of either sex were kept in the laboratory on a diet consisting of 50 percent of lean horsemeat, and 50 percent of Kibbles.<sup>5</sup> Dog 12<sup>6</sup> was maintained on a protein-free diet (12) for a period of two months. A dose of 0.5 to 0.8 cc. per kgm. of carbon tetrachloride (C. P. grade) mixed with an equal volume of Wesson oil was given by stomach tube immediately prior to

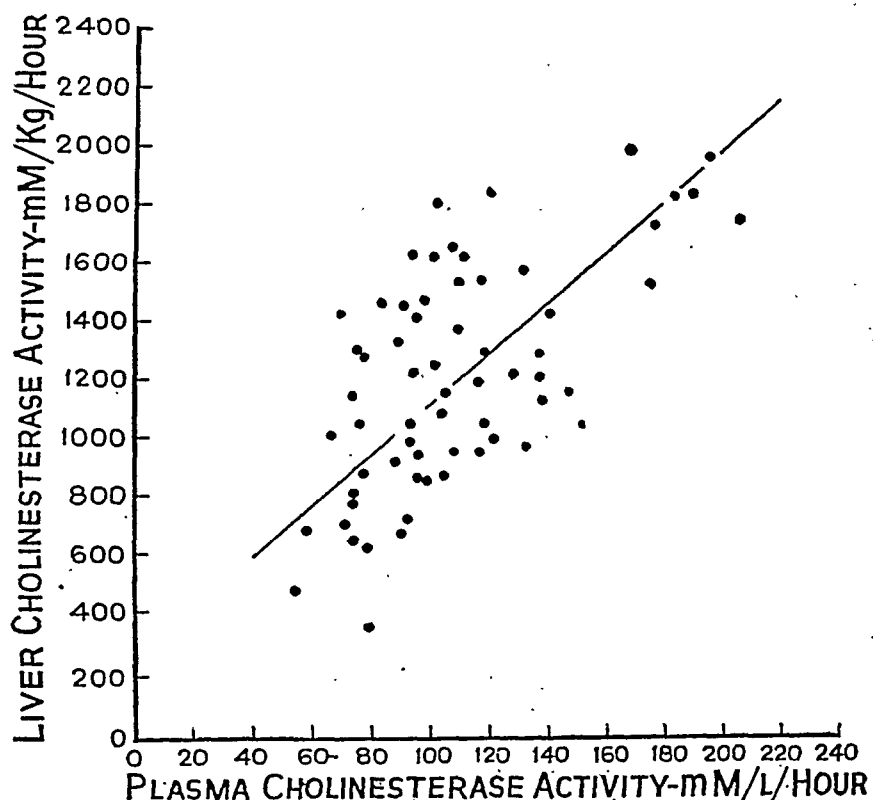


Fig. 1. The relation between the cholinesterase activity of the liver (Y, mean =  $1260 \pm 55$  mM/kgm./hr.) and of the blood plasma (X, mean =  $100.9 \pm 4.6$  mM/L/hr.) of 56 male dogs. The correlation coefficient  $r = 0.398$ ,  $n = 56$ ,  $P < 0.01$ . The regression equation is  $Y = 1260 - 8.39 (X - 100.9)$ .

feeding. This procedure minimized difficulties due to gastric irritation and inadequate absorption. In the chronic experiments this dose was repeated every day or every other day. Plasma cholinesterase activity, serum alkaline phosphatase, hematocrit, and total plasma protein concentration were determined in all dogs on blood samples drawn at suitable intervals, usually just before the administration of the carbon tetrachloride. In several dogs bromsulfalein clearance determinations were also performed.

<sup>5</sup> "Ideal Kibbles", made by Wilson and Company, Inc. Chicago, Illinois.

<sup>6</sup> It was maintained on a protein-free diet for 27 days prior to and during the experimental period, its total plasma protein concentration was stabilized around 4.3 per cent; its plasma cholinesterase activity had not changed significantly between the start of the experimental diet and the start of the period of carbon tetrachloride administration.

The first group of data presented in table 4 shows the effect of carbon tetrachloride administration upon the plasma cholinesterase activity of dogs subjected to this treatment. In all cases a sharp rise in plasma esterase activity followed the liver injury. In the cases of dogs 1, 3, 12, and 18, long continued administration of the drug led to persistent elevation of plasma esterase activity over periods ranging from two weeks to two months.

In the second group of animals shown in table 4 the flow of portal blood from the intestines to the liver had been interfered with prior to the administration of carbon tetrachloride. (Dog 4: total ligation of the portal vein (two stages); dogs 2 and 10: porto-caval shunts between the entrance of the splenic and the gastro-duodenal veins into the vena porta; dogs 7 and 23: complete porto-

TABLE 3

*The effect of prolonged liver damage upon the cholinesterase activity of the liver*

DOG NO.	PLASMA CHOLINESTERASE ACTIVITY	LIVER CHOLINESTERASE ACTIVITY	
Normal dogs			
	<i>mM/L/hr.</i>	<i>mM/kgm.liver /hr.</i>	<i>mM/kgm. body wgt./hr.</i>
4 dogs	75-85	(Mean) 910	(Mean) 28
5 dogs	85-100	(Mean) 1100	(Mean) 33
5 dogs	100-120	(Mean) 1300	(Mean) 37
Dogs with liver damage*			
12	142	750	26
15	103	535	15
18	300	510	18
34	138	720	17

\* Dogs 12, 15, 18 due to  $\text{CCl}_4$ . Dog 34 hepatitis of unknown origin and more than ten days' duration.

caval<sup>7</sup> shunts.) Each dog received two doses of carbon tetrachloride in amounts and under conditions comparable to those employed with animals 12, 15, 19, 28, 32, 33. As expected with a substance of this type (which remains in the body for 48 hrs. or more) some effect was observed in all cases; it was most marked in the two dogs with incomplete porto-caval shunts.<sup>7</sup> In all cases the increases in plasma cholinesterase were less than the increases seen with comparable normal animals.

Figures 2 and 3 present in greater detail the changes observed in two representative dogs. Dog 3 was a female which had been kept in the laboratory for a week before the start of the experiment. It was exposed to 0.7 cc. per kgm. body weight of carbon tetrachloride for 22 days. Its weight dropped from an initial 14.3 kgm. to 13.3 kgm. at the time that the drug was discontinued.

<sup>7</sup> The authors are greatly indebted to Dr. Tague Chisholm, of the Surgical Laboratory of this Medical School. His help and advice were invaluable in the preparation of the porto-caval shunt dogs used in this work.

Two weeks later it had regained its initial weight. Plasma cholinesterase and serum alkaline phosphatase activities showed parallel increases during the production of liver damage; total plasma protein levels dropped and erythrocyte counts increased almost simultaneously. After discontinuation of the carbon tetrachloride the phosphatase activity, the total protein, and the erythrocyte counts recovered more promptly than the cholinesterase activity of the plasma in this as in the other dogs treated over a longer period.

TABLE 4

*The effect of carbon tetrachloride poisoning upon the plasma cholinesterase activity of normal dogs and of dogs with reduced portal circulation*

DOG NO.	DOSE OF CCl <sub>4</sub> CC. PER KGM.	DAYS FROM FIRST DOSE TO MAXIMUM PLASMA CHOLIN- ESTERASE	PLASMA CHOLINESTERASE ACTIVITY mM/L/hr.		PER CENT OF INCREASE	
			Basal	Maximal	Uncorrected	Corrected for hematocrit change
Normal dogs						
1	0.7	3	100	200	100	62
3	0.7	4	80	160	100	62
12	0.8	4	102	172	69	39
15	1.0	3	117	173	48	27
18	1.0	2	141	310	120	78
19	0.8	2	120	200	66	36
20	0.5	4	118	208	76	33
28	0.8	2	112	201	79	44
32	1.0	3	81	178	120	81
33	1.0	1½	69	112	62	38
Mean.....					84 ± 7.7	50 ± 6.1
Dogs with reduced portal circulation						
2	0.7	4	97	142	46	24
4	0.7	4	92	110	19	3
7	0.7	4	107	119	9	11
10	0.8	3	77	117	51	34
23	1.0	3	70	92	32	8
Mean.....					31 ± 7.9	16 ± 5.6

Dog 28 was a male weighing 17.2 kgm. at the beginning of the experiment; it had been kept in the laboratory for 10 days. One dose of 0.8 cc./kgm. of carbon tetrachloride was administered. Samples were taken before that dose, 17, 22, and 40 hours thereafter, and then every day for ten days. The dog lost weight during the first two days after the beginning of the experiment, and never recovered this loss. It did not appear well until after about two weeks. The plasma cholinesterase activity began to rise about 20 hours after the administration of the drug, reached its peak after about 72 hours, and thereafter declined

fairly promptly. The *BSP* elimination decreased before the onset of the increase in the plasma esterase activity, the serum alkaline phosphatase increased somewhat later, and the changes in total protein and in hematocrit appeared still later. The bromsulfalein elimination values agree with the observed plasma cholinesterase activity which indicated an exacerbation of the liver disease about five days

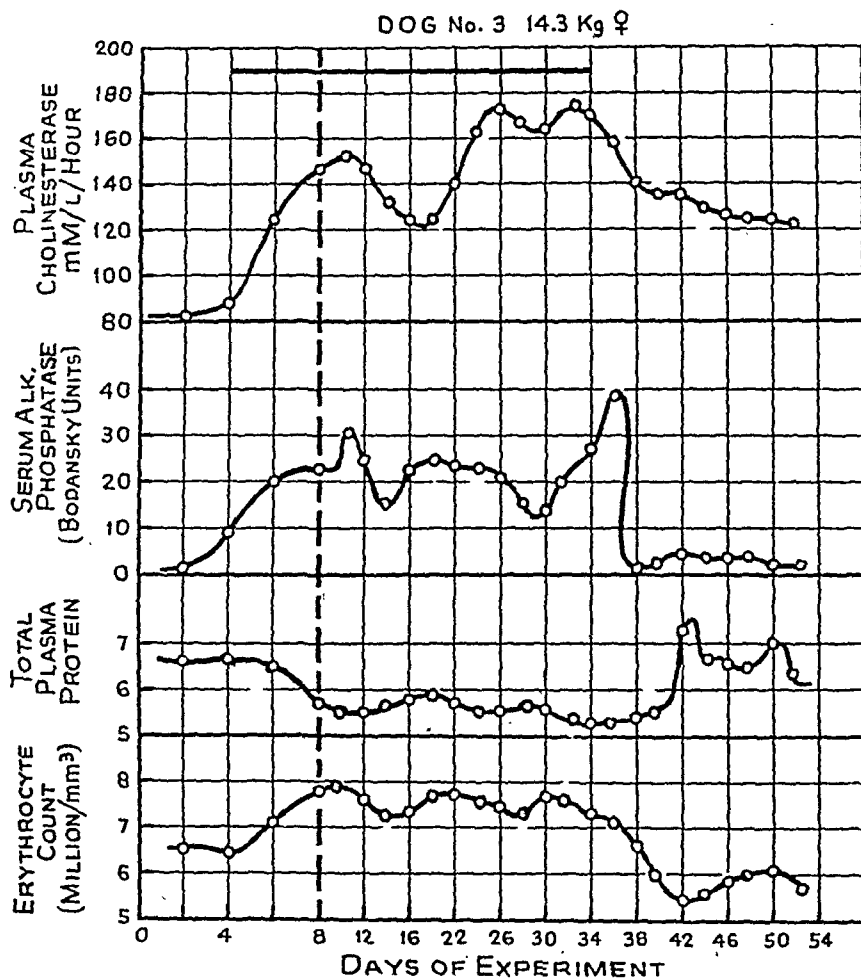


Fig. 2. Findings in dog 3, which was exposed to carbon tetrachloride during the period indicated by the horizontal bar which appears at the top. For details see text.

after the start of the experiment. Recovery toward normal occurred at about the same rate for the bromsulfalein elimination and the cholinesterase values.

The increase in plasma cholinesterase activity following carbon tetrachloride poisoning does not appear to be accompanied by any comparable changes in the cholinesterase activity of the liver. Only after prolonged periods of liver injury is there any evidence of an effect upon the liver esterase content. This is shown by the series of data contrasted with normal values in table 3. It appears that all of the sick dogs showed abnormally low liver esterase values.

Thus, while it is not usually possible to detect deviations from normalcy by the analysis of a single plasma sample, deviations of the type here described as due to liver injury can be detected in many cases by a simultaneous analysis of the cholinesterase activities of plasma and of liver biopsy samples. Insertion

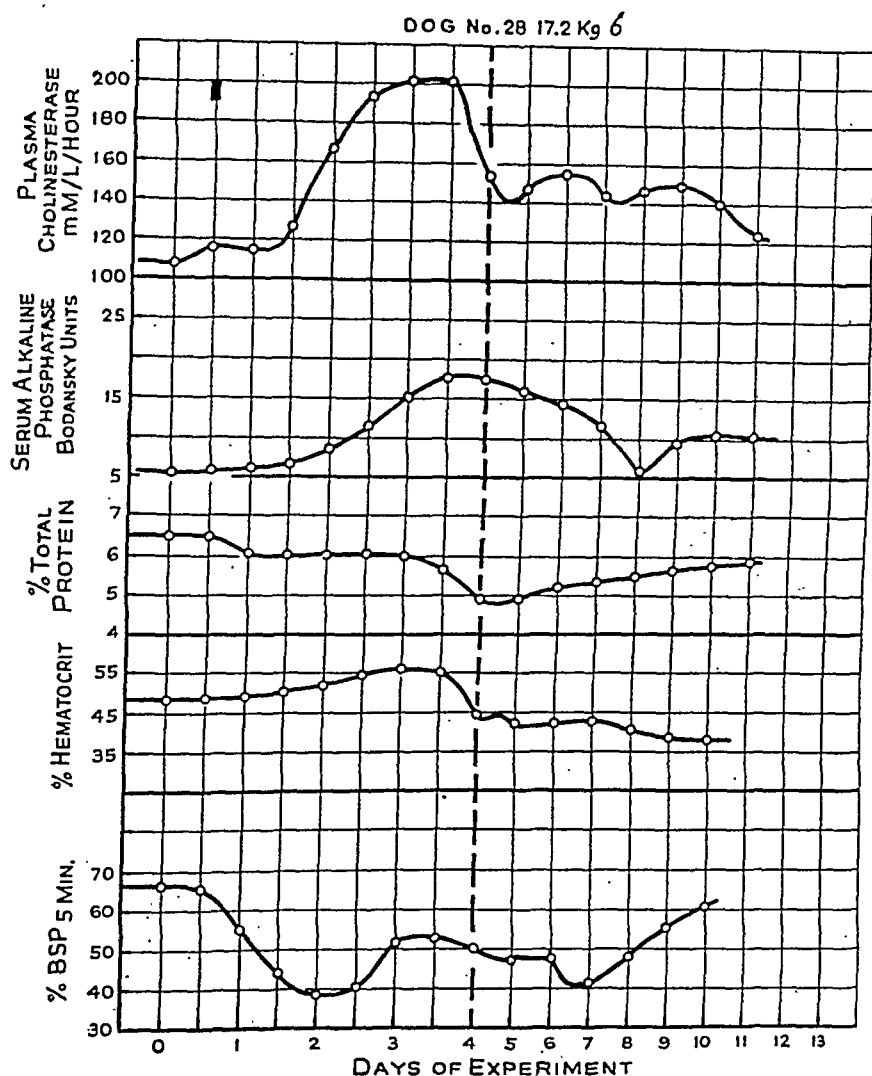


Fig. 3. Findings in dog 28, which was given 0.8 cc./kgm. of carbon tetrachloride at the time indicated by the short vertical line at top. For details see text.

of the values obtained into a diagram similar to figure 1 will, in the presence of acute liver disease, generally yield points to the right and below the normal range.

C. *The effect of changes in plasma volume upon the circulating plasma cholinesterase activity of normal and of "functionally hepatectomized" dogs.* The correlation between liver and plasma cholinesterase activities, and the marked effect of liver injury upon the plasma cholinesterase activity of the dog suggested the possibility of a direct exchange of the enzyme between the liver parenchyma and the blood plasma. Attempts to demonstrate directly the release of esterase

from the liver by the comparison of the cholinesterase activity of systemic and hepatic venous blood in highly acute carbon tetrachloride poisoning failed, probably because of the slow course of the reaction. However, an indirect method did permit us to demonstrate a release of cholinesterase activity from the liver into the blood stream.

Since crystalline human or bovine albumin<sup>8</sup> contains only traces of plasma cholinesterase (13a) and since (14) the transfusion of concentrated albumin solutions into patients or animals leads to an increase of the plasma volume which is long lasting (especially following hemorrhage) it is possible to effect an artificial reduction of the plasma cholinesterase level in experimental animals by such transfusions.

Mongrel dogs of either sex were kept in the laboratory for at least one day prior to the experiment in order to assure an adequate degree of hydration. All experiments were performed under sodium pentobarbital anesthesia, the level of anesthesia being maintained approximately constant by the injection of additional anesthetic at regular intervals. Blood samples were taken from the left femoral vein, all injections were made into the right femoral vein; the total volume of blood withdrawn for analysis during the course of an experiment amounted to 40 to 50 cc. After a preliminary plasma volume determination, a volume of blood ranging from 80 to 140 cc. was rapidly withdrawn from the right femoral artery. A 25 percent solution of crystalline albumin—either human or bovine—was injected in amounts of 20 percent of the total volume withdrawn. The albumin preparations employed were analyzed and found to contain either no cholinesterase activity at all, or only traces which invariably amounted to less than 3 percent of an equal volume of dog plasma, i.e., less than about 0.6 percent of an equivalent volume of plasma. Blood samples were then taken every half-hour for several hours and a second determination of plasma volume was performed between 90 and 120 minutes after transfusion. The hematocrit, the total protein content, the amount of dye present, and the plasma cholinesterase activity were determined in each sample.

Three other series of experiments were performed which differed from the one just described in the use of splenectomized, or eviscerated (15), or "functionally hepatectomized" dogs in place of the intact animals. The functional hepatectomy was performed on animals with chronic or acute portocaval shunts (established by means of the technique of Blakemore and Lord (16)) by complete ligation of the gastro-hepatic ligament close to the porta hepatis. In all animals which had undergone surgery, a period of one hour elapsed between the completion of the operation and the beginning of the experiment.

The albumin transfusion in all cases was followed by an increase in plasma volume as indicated by T 1824 measurements (table 5). In all but the series

<sup>8</sup> The authors wish to thank Dr. E. J. Cohn and Dr. L. Strong, Department of Physical Chemistry, Harvard Medical School, and Dr. J. B. Lesh, Armour Research Laboratory, Chicago, Illinois, for placing at their disposal the human and the bovine crystalline albumin, respectively, used in these experiments.

TABLE 5

*The effect of transfusions of concentrated plasma albumin upon the plasma cholinesterase activity of the dog*

DOG NO.	PLASMA VOLUME				PLASMA CHOLINESTERASE ACTIVITY			
	Before	After	Increase due to albumin transfusion	Before	After	Decrease due to transfusion	Increase in total circulating cholinesterase activity	
	Bleeding			Transfusion				
Intact dogs								
18*	cc.	cc.	%	cc./g. albumin	mM/L/hour	mM/L/hour	%	%
37	480	420	33	18.7	76	71	7	24.7
38	505	435	32	18.7	73	65	11	21.8
43	510	414	36	20.1	93	92	1	35.8
54	590	520	22	23.0	87	83	5	16.2
55	300	230	34	11.3	116	112	4	30.0
Average .....			32.5	18.4			4.8	27.8
St.d.m.†.....			±2.3	±1.9			±1.2	±3.4
Splenectomized dogs								
62	392	345	28	19.0	106	93	11	12.0
63	495	445	24	19.6	195	192	2	22.0
64	370	295	48	19.2	88	77	13	30.4
65	285	235	60	22.5	114	89	22	23.5
Average.....			40.0	20.1			12.0	22.0
St.d.m.....			±7.5	±2.6			±4.1	±3.9
Hepatectomized dogs								
2	390	320	22	9.4	115	91	21	-3.5
7	615	490	37	15.8	88	65	26	2.0
10	600	575	18	13.4	93	80	14	1.0
23	595	570	19	22.0	72	66	8	8.8
26	650	620	23	23.4	83	68	18	1.2
51	270	230	31	14.0	88	71	19	5.2
56	380	360	31	14.8	99	77	22	2.0
67	570	550	18	17.0	82	72	12	3.9
68	598	530	27	18.6	75	60	20	1.8
Average.....			25.2	16.35			17.8	2.5
St.d.m.....			±2.3	±1.4			±1.9	±1.1
Eviscerated dogs								
44	290	268	24	14.2	105	93	13	4.0
48	165	132	60	15.8	92	61	33	7.8
49	372	352	16	8.9	84	75	11	4.2
50	290	265	21	8.9	87	70	18	-1.0
Average.....			30.3	11.9			19.9	3.5
St.d.m.....			±10.2	±1.8			±4.9	±2.1

\* Dog 18 had been exposed to carbon tetrachloride and still showed evidence of severe liver damage; for this reason plasma volume changes were calculated from the hematocrit values.

† Standard deviation of the mean.

of eviscerated animals this observed increase came close to that predicted on the basis of available data concerning the osmotic properties of canine plasma and of the albumin preparations employed (17), one gram of albumin calling forth 17-20 cc. of plasma. In the eviscerated animals considerably smaller increases were noted, i.e., less water was drawn into the blood stream in these preparations.

Hematocrit values closely reflected the extent of the plasma volume changes in all but the liverless animals. In the latter, occlusion of the hepatic artery should be followed within one or two hours by severe hypoglycemia accompanied by a sympatho-adrenal discharge which would induce contraction of the spleen. Thus, a quantity of blood containing a large excess of erythrocytes would be injected into the blood stream of the liverless animals. On the basis of available information concerning the capacity of the spleen, the quantity of blood thus discharged could be quite enough to account for the observed discrepancies between the plasma volume changes indicated by the hematocrits and by the Evans blue values respectively. This interpretation is made the more likely by the finding of an intensely contracted spleen within ninety minutes after the operation in the hepatectomized animals, while the intact animals retained the enlarged spleen commonly observed under nembutal anesthesia; furthermore, the eviscerated animals which did not have a spleen, did not show any such effect.

Thus, in all of the animals of these series, increases in plasma volume averaging near 30 percent were brought about without the introduction of any plasma cholinesterase from outside sources. This dilution of the pre-existing plasma esterase in all cases resulted in an initial decrease in the plasma esterase activity of the animals so treated.

In the normal and splenectomized dogs, this initial drop of plasma cholinesterase activity was followed by gradual recovery. In the eviscerated and in the liverless dogs, a more pronounced initial fall occurred and low cholinesterase activity persisted without evidence of recovery after two hours. The resulting changes in the amount of circulating cholinesterase are summarized in the last column of table 5. In those animals which no longer had an intact liver the amount of cholinesterase activity in the circulation remained substantially constant. By contrast, the splenectomized as well as the intact dogs, following a transfusion of concentrated albumin solution, responded with a marked increase in the circulating cholinesterase, an increase which must reflect a transfer of esterase from some store to the blood stream. Since in the absence of a circulated liver such a store is not in evidence, it would appear that the liver is the most plausible site in which to localize this mobile store.

**DISCUSSION.** While it has been shown that the amino acids of all plasma proteins appear to be turned over at a rapid and fairly uniform rate, the conclusion does not appear warranted that the various plasma proteins do not behave as individuals, and that their plasma concentrations are not controlled by factors peculiar to each one of them, more or less independently of one another.

A study of these factors requires methods for the identification—the tagging—not of individual amino acids, but of a whole protein molecule; so that one species can be traced and studied. The present report concerns one such instance in which the enzymatic activity of the protein in question was utilized as the labelling characteristic.

Plasma cholinesterase is a normal component of dog, as of human blood plasma. In spite of wide variations from dog to dog, the plasma cholinesterase activity of individual healthy animals remains relatively unchanged over considerable periods of time.

Mendel and co-workers (3) have shown that the greatest part of the cholinesterase activity of dog plasma determined as in the present study is due to the soluble enzyme here referred to as plasma (cholin-)esterase. Using  $\beta$ -methyl acetylcholine as a substrate, plasma samples from normal as well as from carbon tetrachloride treated dogs have been examined for their content of the cholinesterase characteristic of nervous tissue and of erythrocytes; however, no more than traces of activity have been detected in any case. A study of the distribution of the cholinesterase activity among the fractions of human blood plasma (13) has been undertaken and it has been shown that 80 to 90 percent of the total activity can be concentrated in a small subfraction of the fraction IV, 4 of Cohn *et al.* These findings are being duplicated on dog plasma.<sup>9</sup> The cholinesterase activity of canine blood plasma, therefore, is due either to a single protein, or to several which have similar chemical and physical properties. The best preparations of human plasma cholinesterase have been found electrophoretically to consist largely of  $\alpha$ -globulins (18b).

Plasma cholinesterase activity in dogs varies independently of the total protein concentrations. This was observed on statistical examination of the results obtained with 44 dogs, and it was confirmed by studies of dogs with carbon tetrachloride poisoning as well as by some preliminary studies concerning protein depleted dogs. If, as suggested above, plasma cholinesterase is part of the  $\alpha$ -globulin complex, then these results are in good accord with those of other workers who have shown (18) that in protein depleted dogs low plasma albumin and total protein concentrations can be attained without a concomitant drop in the  $\alpha$ -globulin level. One implication of these results is that it is unlikely that present methods of protein depletion will be applicable as such to the depletion of the cholinesterase reserves in dogs.

The site of origin of this enzyme is yet uncertain. Data to be presented elsewhere indicate that an analogy with antibody proteins is not admissible, that the lymphoid tissues are not responsible for the production of plasma cholinesterase. Attempts at depletion have so far not been successful. Arguing by analogy from the rat and man there is some reason to believe that in the dog, too, the liver is the main site of plasma cholinesterase production.

Certainly the liver is an organ exceptionally rich in plasma cholinesterase. Gram per gram it is second only to the intestinal mucosa. The enzymatic

<sup>9</sup> The authors are indebted to Dr. J. B. Lesh, Armour Research Laboratory, Chicago, Illinois, for this information as well as for undertaking the fractionation of dog plasma.

properties of liver cholinesterase—with regard to solubility, to substrate specificity, and to kinetics—indicate that only small amounts of the nerve and erythrocyte type of cholinesterase are present, and other data (19) make it likely that the bulk of the activity measured is not due to ordinary “liver esterase”. Final proof of the presumed identity of the enzymes responsible for the cholinesterase activities of liver and of blood plasma must await the successful fractionation of the proteins of the liver.

The correlation between liver and plasma cholinesterase activities suggests a physiological equilibrium between the hepatic parenchyma and the plasma. As a consequence the entire cholinesterase activity of the liver can be conceived of as a reservoir of potential plasma cholinesterase which exists preformed in that tissue. The magnitude of this reservoir can be calculated as follows:

Ratio $\frac{\text{mean liver}}{\text{mean plasma}}$ cholinesterase activities (fig. 1).....	12.3 cc./gram
Mean liver weight per kgm. body weight (16 dogs).....	23.5 gram
Mean plasma volume per kgm. body weight (20).....	47.0 cc.
Ratio $\frac{\text{mean liver}}{\text{mean plasma}}$ cholinesterase activity per kgm. body weight..	6.2

These considerations also suggest that the increase in plasma cholinesterase activity following carbon tetrachloride poisoning represents a release of part of the cholinesterase store from the liver, perhaps comparable to the release of protein observed in heat injured, perfused livers (21). The arguments for and against such an assumption can be summed up as follows.

Carbon tetrachloride under the conditions employed in this work will not produce any demonstrable degree of extrahepatic injury until it has been administered a considerable number of times (22). Since the esterase increase occurs after one or two doses of the poison, damage other than liver damage can hardly be involved in this phenomenon. The experiments on animals with occluded portal veins also support this conclusion, since here the ratio of hepatic to extrahepatic injury was greatly decreased, and simultaneously much smaller cholinesterase increments were seen.

At one time hemoconcentration as indicated by increased hamatocrit values was considered an integral part of liver disease. While recent data (23) have cast serious doubt on this assumption, table 5 includes values for the increments of circulating cholinesterase following carbon tetrachloride administration. These values are calculated on the assumption that the erythrocyte volume had remained constant, the entire change of the hematocrit values being due to changes in plasma volume. In spite of this “correction” substantial plasma esterase increases remain in the group of intact dogs.

Changes in specific activity of the cholinesterase, rather than in its absolute amount, might occur. It could be shown that dialyzable activators were not present in the plasma of carbon tetrachloride treated dogs and that mixed plasma samples from normal and from poisoned dogs showed strictly additive activities. This possibility is not completely ruled out by these experiments.

The increased plasma cholinesterase might reflect a breakdown of destruction or excretion processes in which the liver might play a major rôle. However, the establishment of a porto-caval shunt (24) in dogs leads to a decrease, not an increase, in the plasma cholinesterase activity, and biliary excretion cannot account for more than a minute fraction of the observed changes (gall bladder bile in dogs contains no more than 5 percent of the cholinesterase activity of an equal volume of plasma; bile salts do not affect the activity of the enzyme (2)).

Thus, it appears most likely that the increased plasma cholinesterase activity of carbon tetrachloride treated dogs is due to a transfer of enzyme from the liver into the blood stream. Whether this is accompanied by an increased rate of production of the enzyme or whether it reflects merely a permeability change accompanying the injury cannot now be decided.

It was, however, possible to demonstrate by means of the indirect method outlined in part C of this paper, that a fraction, at least, of the hepatic cholinesterase reserve is readily mobile. Thus an extra 25 percent of cholinesterase can, under certain circumstances, be added to the blood plasma in less than two hours. The exact nature of the stimulus capable of eliciting such a cholinesterase release is not yet clear. Several experiments have indicated that simple dilution may not be enough, but that the presence of an elevated plasma albumin level may be required for the release of extra esterase. At any rate, however, two things have been demonstrated here: a preformed store of an enzyme very similar to plasma cholinesterase exists, and this store is localized largely, if not entirely in the liver. It seems likely that cholinesterase is not the only one of the plasma proteins for which such a mobile reserve exists (24). If it could be shown that such stores in other instances, too, are located in the liver, then it will again become necessary to consider the possibility that certain of the liver cell proteins are in direct equilibrium—as such—with similar proteins in the blood plasma.

#### SUMMARY

1. Plasma cholinesterase activity in the dog was found to be independent of sex and of total plasma protein concentration.

2. A significant degree of correlation between liver and plasma cholinesterase activities of male dogs was found. Calculation showed that the liver contains five to seven times the amount of the cholinesterase activity circulating in the blood plasma.

3. Carbon tetrachloride poisoning of dogs results in an increase in the plasma cholinesterase activity. An experimental analysis showed that this effect is due to the liver injury produced, that it is due to an actual increase of circulating plasma cholinesterase amounting to about 50 percent above normal, and that it does not reflect a failure of destruction or excretion of this enzyme.

4. The effect of transfusions of concentrated serum albumin solutions upon the plasma cholinesterase activity of intact, splenectomized, eviscerated, and functionally hepatectomized dogs was examined. It was shown that a decrease

of the plasma cholinesterase activity due to dilution follows the transfusion, and that in the presence of a functioning liver this fall in plasma esterase activity is followed by the release of additional enzyme into the blood, restoring the esterase level to normal in about two hours with an increase in the amount of circulating enzyme. No such effect could be detected in eviscerated and in functionally hepatectomized dogs.

5. The data have been interpreted as indicating the existence of a considerable store of preformed plasma cholinesterase in the liver of the dog; an equilibrium seems to govern the relation between liver and plasma cholinesterase activities; at least 25 per cent of the circulating plasma cholinesterase activity can be rapidly replaced from this hepatic store.

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# STUDIES ON SKIN TEMPERATURE AND CIRCULATION IN DECOMPRESSION SICKNESS<sup>1</sup>

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The quantity of dissolved gases in the body is generally assumed to be related to decompression sickness. The rate of nitrogen elimination from the body determines the amount of dissolved nitrogen ultimately present in a supersaturated state following decompression. Since the nitrogen elimination of the tissues is limited by the tissue blood supply, it is important to study the relationship of the vascular bed to the problem of decompression sickness.

Studies of exchange of radioactive inert gases in the extremities, especially krypton, have verified the above statements regarding nitrogen exchange (1). There was increased elimination of krypton from the hand following its direct warming, or the warming of another extremity. The latter brought about presumably reflex dilatation in the vessels of the hand measured. Cooling of the hand decreased the rapidity of inert gas exchange, whereas exercise or diathermy increased the inert gas exchange. Cook and Sears (2) have shown that in nembutalized dogs caffeine and theophyllin also increase the rate of inert gas exchange in the leg. Measurements made of the vasomotor response under conditions associated with chamber decompression show a close parallelism.

The decrease of circulation in limbs affected with pain and the subsequent drop in skin temperature are familiar phenomena in decompression sickness; the vasomotor reaction to unrelieved pain may often lead to secondary shock. Whether or not the state of the vascular bed during the initial phases of decompression has any bearing on the subsequent development of decompression sickness symptoms was not known near the end of 1942, when the present investigation began.

A number of observations has been reported bearing upon vascular phenomena in connection with decompression sickness. Knisely (3) has reported that there is frequently vasoconstriction of the scleral vessels in subjects that are being decompressed and that these same subjects are likely to develop symptoms of decompression sickness. Later he and his collaborators (4) presented some evidence to the effect that a reduced rate of blood flow through the tissues of a part may contribute to the cause of bends pain. In 36 of their subjects, who had arms or legs held up vertically while at altitudes above 30,000 feet, elevation of the extremity either initiated bends pain, or, if pains were already

<sup>1</sup> This work was carried out in 1943 under a contract between the Office of Scientific Research and Development and the University of California. The following persons collaborated in securing the data herein reported: F. M. Henry, W. R. Lyons, H. B. Jones, W. N. Sears, S. F. Cook and J. B. Mohney.

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present, made them more severe. In subjects that had previously received aminophyllin, elevation of the part usually did not bring on pain.

Preliminary measurements by F. M. Henry (5) on young male subjects showed a decrease of skin resistance during altitude chamber flights. The decrease of skin resistance preceded by a significant period of time the onset of discernible pain of decompression sickness; frequently the decrease occurred during the ascent. The Yale Aeromedical Unit (6) observed a decrease of digital blood flow during ascent to 38,000 feet and 100 minutes of subsequent chamber flight. Measurements were done by the Nyboer impedance technique. Fraser (7) found that a subject with a circulatory abnormality resembling Raynaud's disease was extremely susceptible to decompression sickness. He developed incapacitating pain in the extremity involved with the disease.

As far as the effect of ambient temperature on development of decompression sickness symptoms is concerned, Fraser and Rose (8) have demonstrated that the incidence of decompression sickness increases with the lowering of environmental temperature. Anthony et al. (9) and Griffin et al. (10) have also arrived at the conclusion that during simulated ascents in the decompression chamber men kept comfortably warm suffer significantly less from joint bends than they do when they are definitely cold. The data show this effect most clearly for subjects, at rest, when their subjects exercised, the difference in performance was not very definite. Smedal et al. (11) have published results indicating that in a short (20 min.) simulated ascent with moderate exercise more subjects have bends pain at room temperature than in the cold altitude chamber.

The rate of inert gas exchange at 38,000 feet in the hands of five subjects was measured by us using the *in vivo* radioactive technique. In three of these gas exchange was slowed down, decreased circulation in the hand thus being indicated. Because gas exchange experiments were unsuitable for rapid determinations of circulation rate on a large number of subjects in the altitude chamber, a series of experiments was then carried out in which the skin temperature of the dorsal surface of the hand of the subjects was measured in order to obtain information regarding the state of their surface blood supply.

A radiometer, somewhat improved<sup>3</sup> over the one described by Gier and Boelter (12), was used. This instrument has a silver plated constantan thermopile as a sensitive element. The radiometer was suspended on a moving arm from the ceiling of the altitude chamber and was moved by the operator from one subject to the next. Readings were made by another operator seated outside of the chamber.

At most, eight subjects were in the altitude chamber at a given time. Their hand temperatures were measured at 10-minute intervals throughout the entire simulated ascent. In spite of the fact that under certain conditions the radiometer was sensitive enough to measure temperatures with an error of less than 0.1°C., we were not able to avoid larger errors in the actual series of measurements for several reasons. First, there was a change in calibration of the radiometer due to the reduced barometric pressure and the increased humidity.

<sup>3</sup> To be described in a separate publication.

Tests showed that no more than 0.2°C. error was caused by this effect. Secondly, the cold junctions of the thermopile shifted their values during each set of measurements. This error was also corrected, but it raised the inaccuracy of a single observation to 0.5°C. Errors resulted also from the fact that the subjects did not expose the same area of their hands to the radiometer. It was thus decided to give little attention to individual temperature records, but rather to study the distribution of certain groups as a whole.

The rates of ascent simulated the flight of bomber planes to 35,000 feet. Standard step up exercises were carried out at five minute intervals, and a self-rated nine-point scale was used as a measure of decompression sickness pain. Altitude chamber procedures have been reported previously (13).

TABLE 1  
*Mean dorsal skin temperatures of the left hand*

TIME FROM START OF ASCENT	ALTITUDE	NO. OF EXERCISES	GROUP A THOSE WITH NO PAIN			GROUP C THOSE WITH SEVERE PAIN				
			No. of subjects	Mean skin temp.	Standard deviation of mean	No. of subjects	Mean skin temp.	Standard deviation of mean	No. of subjects with pain	Mean pain intensity
<i>minutes</i>	<i>feet</i>			°C.			°C.			
0	0		23	32.77	0.24	26	32.44	0.33	0	0
32	28,500	2	24	33.40	0.22	26	32.43	0.28	4	0.15
42	32,600	4	24	33.29	0.21	26	32.15	0.28	6	0.31
52	35,000	6	24	33.55	0.21	25	32.16	0.31	14	1.8
62	35,000	8	24	33.42	0.23	22	32.12	0.36	17	3.2
72	35,000	10	24	33.20	0.25	13	31.80	0.43	11	3.1
82	35,000	12	24	33.48	0.25	10	31.87	0.47	8	2.5
92	35,000	14	24	33.47	0.22	9	32.15	0.44	8	3.6
102	35,000	16	24	33.41	0.21	8	31.70	0.40	8	5.1
112	35,000	18	24	33.17	0.20	7	30.99	0.44	7	5.1
122	35,000	20	24	33.05	0.21	4	31.38	0.28	4	5.8

*Skin temperature data.* For purposes of comparison the subjects were divided into three groups: Group A consisted of 24 subjects who did not develop any bends pain or chokes in the particular chamber run; 16 subjects, those with mild symptoms (pain intensity 1 to 3) formed group B; and 26 subjects were in group C. These latter had severe or incapacitating bends or chokes symptoms (pain intensity equal to, or higher than, four).

First, the mean temperatures and their standard deviations were computed for each group (table 1). The skin temperatures of the dorsum of the hand for subjects of group A increased, though not significantly, during ascent in spite of the fact that the chamber air temperature dropped.<sup>4</sup> This increase could possibly be explained by taking into account the fact that the presence of several subjects in the chamber may raise the effective radiation temperature com-

<sup>4</sup> The chamber runs were carried out without temperature control; there was no air conditioning to keep the temperature constant during ascent. On the average this increase was from an inside temperature of 72° to 80°F.

pared to the outside air by about two degrees. The skin temperatures adjust themselves to such changing conditions slowly. At maximum altitude the mean skin temperature of the dorsum of the hand remained essentially constant in group A. At ground level, just before the chamber test was started, the mean skin temperature of group C did not significantly differ from that of groups A or B. As low as 28,000 feet the mean difference amounted to  $0.97^{\circ}\text{C}.$ , signifying that these subjects would later develop bends pain. Thereafter the mean skin temperatures of group C remained significantly below those of group A during the whole chamber run (see table 2).

In order to distinguish a temperature drop secondary to pain from one that might have been present before even "barely perceptible" (grade 1) pain was sensed by the subjects, we analyzed the temperature readings of group C a few

TABLE 2  
Mean skin temperature differences between groups "A" and "C"

TIME FROM START OF RUN	ALTITUDE	NUMBER OF EXERCISES	MEAN NUMBER OF SUBJECTS	$t_a - t_c$ MEAN SKIN TEMP. DIFFERENCE	STANDARD DEVIATION OF MEAN DIFFERENCE	CRITICAL RATIO	PROBABILITY $P$
<i>minutes</i>	<i>feet</i>						
0	Ground level		24	+0.33	0.41	0.8	>>5%
32	28,500	2	25	0.97	0.36	2.72	5% > P > 1%
42	32,600	4	25	1.14	0.34	3.32	<1%
52	35,000	6	24	1.39	0.37	3.74	<1%
62	35,000	8	23	1.30	0.42	3.07	<1%
72	35,000	10	18	1.40	0.50	2.82	5% > P > 1%
82	35,000	12	17	1.61	0.54	3.01	5% > P > 1%
92	35,000	14	16	1.32	0.49	2.68	5% > P > 1%
102	35,000	16	15	1.71	0.45	3.88	<1%
112	35,000	18	15	2.18	0.48	4.55	<1%
122	35,000	20	14	1.67	0.35	4.78	<1%

minutes before pain started. The mean temperature was  $1.09^{\circ}\text{C}.$  lower than the mean temperature of subjects of group A. When these subjects had mild pain, this drop increased to  $1.79^{\circ}\text{C}.$ ; and, when they developed severe pain, their mean skin temperature significantly dropped  $2.21^{\circ}\text{C}.$  below that of group A (see table 3). After the subjects became incapacitated the measurements were sometimes continued in the chamber lock during descent. Low skin temperature and "cold sweat" were almost universal during this period, the fall in skin temperature correlating closely with the degree of systemic reaction (sometimes approaching vasomotor collapse) when the subjects left the chamber.

The mean skin temperatures of group B lay between those of groups A and C. The difference in temperature between groups A and B was not statistically significant.

The skin temperature of 19 subjects was measured on two consecutive chamber runs, in addition to two classification chamber runs where these men were

classified as belonging to "relatively susceptible" and "relatively resistant" groups. The average of the two temperatures taken was compared with the classification grouping of these subjects. There was no demonstrable relation between resistance or susceptibility to bends and skin temperature if the skin temperatures were taken at ground level or early during the chamber test. Classification as to resistance or susceptibility to bends cannot be attained by measuring dorsal skin temperatures of the hand in short chamber runs.

It was of interest to plot the measurements taken on various individuals in the course of this work. In diagram 1, F and G represent measurements on four individuals in group A. Their temperatures all seem to run high, but they are not free of fluctuations, some of which may have been due to experimental error. A, B, C, D, and E are plots of individuals who belong to group C. In

TABLE 3

*Mean skin temperature difference between those with no pain (group "A") and those with severe pain (group "C") before pain started, with mild and severe pain*

DESCRIPTION	NO. OF SUB- JECTS	MEAN TEMP., $t$	STAND- ARD DEVI- ATION OF MEAN	MEAN TIME OF MEAS- URE- MENT	MEAN PAIN INTEN- SITY	MEAN TEMP. DIFFER- ENCE $t_a - t_c$	STAND- ARD DEVI- ATION OF THE MEAN	CRITI- CAL RATIO	PROBA- BILITY
		°C.		minutes		°C.			
Group "A"—Mean tem- perature at altitude.....	24	33.34	0.22		0				
Group "C"—Last measure- ment before subjects re- ported pain.....	26	32.25	0.28	46	0	1.09	0.36	3.06	<1%
Group "C"—Onset of pain.	26			53					
Group "C"—Measurement with mild pain.....	20	31.55	0.32	57	2	1.79	0.39	4.64	<<1%
Group "C"—Measurement with severe pain.....	24	31.13	0.23	80	5.2	2.21	0.32	6.96	<<1%

addition to their temperature, a record of the onset and severity of their pain is shown by the shaded area. The times at which they were forced to descend are also indicated. Some of these plots illustrate the relation of the vasomotor state to the severity of pain. Others are interesting in that the fall in skin temperature began before the appearance of the pain of bends.

*Diathermy treatments.* As a different approach to the problem of circulation in decompression sickness, a study of the effects of heat applied locally by means of short wave diathermy was undertaken. With this apparatus it is possible to cause the opening of the capillary bed and to elevate considerably the temperature of the deeper tissues without increasing the body temperature markedly or causing uncomfortable overheating of the skin. The cable of a Fisher diathermy apparatus was wrapped five times around the left knee of each subject. A similar coil, with no current flowing, was placed around the right knee. Suitable padding was applied between the skin and the coil. The knee was then

preheated for ten minutes prior to ascent, and the diathermy continued for the duration of the simulated ascent. The rate of ascent was 3,000 feet per minute, and the subjects stayed for two hours at 38,000 feet. They performed an exercise

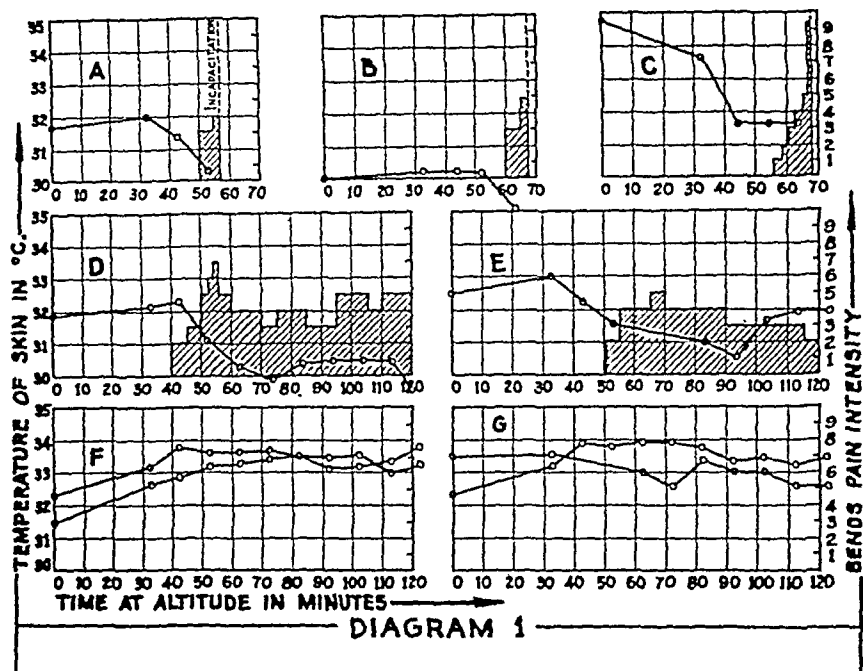


Diagram 1. Typical dorsal surface temperatures of the hand, taken in the decompression chamber at 35,000 feet altitude in function of the time spent there. A, B, C, D, E represent subjects that had bends pain. The shaded areas show the time dependence and subjectively reported intensity of this pain, as well as incapacitation (dotted vertical line). F and G show temperature curves of four subjects that had no symptoms whatever during the test.

TABLE 4  
*Incidence of bends pain in the experiments with diathermy*

	LEFT—LEG, ANKLE, KNEE, HIP,	RIGHT—LEG, ANKLE, KNEE, HIP,	ELSEWHERE AND CHOKES	NO SYMPTOMS	TOTAL NUMBER OF CASES
Control runs.....	61.8%	29.4%	41.2%	14.7%	34
Treatment runs, diathermy on left knee.....	14.7%	53.0%	26.5%	41.2%	34
Critical ratio .....	4.6%	2.0%	1.3%	2.5%	
Probability.....	<1%	>5%	>5%	<5%	

consisting of five deep knee bends every ten minutes; no arm exercises were carried out. Each subject participated in two ascents: one with diathermy (T) and a control (C).

Table 4 shows the incidence of all definite symptoms in 34 subjects. The critical ratios were computed by comparing each group of symptoms in the C and T runs. The decrease in the number of subjects having left leg symptoms

in T runs as compared to C runs is very significant. The change in the incidence in the right leg or elsewhere is not significant, while the number of persons having no symptoms is significantly greater in the T runs than in the C runs.

Further analyses on the time of appearance of various symptoms were carried out. First, subjects were chosen that developed left leg symptoms in either the C or T run. The time of appearance of first degree pain in the left leg, expressed in minutes, was taken as a score. Whenever a subject developed third degree pains elsewhere before his left leg symptoms appeared, this time was taken as the score. The distribution of the scores so computed was then compared in the C and T runs and the mean improvement in the left leg score was 33.4 minutes in favor of the treatment runs. The mean difference divided by the standard deviation of the mean difference gave the critical ratio ( $r = 4.75$ ), which, with this number of cases ( $n = 22$ ) is very significant ( $P < 1$  per cent).

In a similar analysis one finds that the mean of the time score for the first appearance of any first degree symptoms is 47 minutes for C runs and 74 minutes for T runs, with a mean difference of 27 minutes in favor of the T runs. The critical ratio of the difference is 3.19 and is very significant ( $P < 1$  per cent). Thus, there is a general improvement due to the treatment, manifested by later onset of symptoms and by fewer subjects having symptoms. The improvement, however, is very significant only for the mild first degree symptoms; very susceptible subjects fail to show the same improvement. This is reflected by the statistical analysis of scores expressed in terms of percent;<sup>5</sup> the mean score of control runs was 52.8 per cent, of T runs 67.8 per cent, with a critical ratio of 2.17 for the 34 subjects. The probability is somewhat lower than 5 per cent.

The coil was placed on a few subjects after they had developed mild first degree bends pain. None of these cases showed any improvement.

The diathermy treatment did not increase body temperature as measured orally.

*Heated suit tests.* Electrically heated flying suits of the Army Air Force were used. These were originally designed to protect from cold. While wearing these suits, the subjects were comfortably warm throughout the whole chamber run. Oral temperatures were taken on many of the subjects, and usually there was a rise of about one degree above normal. The chamber temperatures in the various runs were substantially constant. Sixteen of our most susceptible subjects were tested, all having had at least three previous chamber runs. The routine University of California chamber test was used (13). The mean score was 29.4 per cent for the three regular runs and increased to 50 per cent when the subjects wore heated suits. The statistical significance can be measured by the critical ratio ( $t = 2.5$  in this case) which falls between the 5 per cent and 1 per cent level in this small group of subjects. It is possible that a suit with more peripheral distribution of heating would yield better results. Some of the subjects complained of feeling extremely fatigued for twenty-four hours after the chamber tests in heated suits.

<sup>5</sup> A scoring method developed by Cook et al. taking both time and intensity factors of bends pain into consideration. One hundred per cent score means a completed altitude chamber test with no symptoms.

## SUMMARY

1. Variation in circulation to the extremities is an important factor in the development of decompression sickness.

2. The dorsal skin temperature of the hand of 24 subjects was measured during simulated ascents to 35,000 feet at room temperature. It was found that the mean temperature of subjects that did not develop decompression sickness remained relatively high and constant throughout the test. Those that developed bends pain had significantly lower hand temperatures prior to the onset of pain. The temperature dropped further after the pain appeared.

3. Diathermy applied to the left knee significantly decreased the incidence and severity of symptoms in that knee and resulted in a slight general improvement of performance. Diathermy applied after onset of bends pain had no effect.

4. Heated suits slightly reduced the incidence of severe bends.

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<sup>6</sup> Microfilm copies of these reports are available at the Office of the Publication Board, Department of Commerce, Washington, D. C.

<sup>7</sup> Copies of these reports are available at the Canadian National Research Council, Institute of Aviation Medicine, 1107 Avenue Rd., Toronto, Ontario, Canada.

# CARDIAC OXYGEN METABOLISM AND CONTROL OF THE CORONARY CIRCULATION<sup>1,2</sup>

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A recent study of the coronary circulation in the dog's heart *in situ* (7) (8) led us to conclude that although these vessels are not under vasomotor nerve control they are dilated by various chemical agencies including oxygen-lack, hydrochloric acid, acetylcholine, and epinephrine. Since coronary ischemia, produced by brief occlusion of the coronary artery, also led to temporary coronary vasodilatation and 100 per cent oxygen inhalation to significant coronary constriction we concluded that these vessels might be capable of automatic adjustment of their tonus to the metabolic requirements of the heart muscle. The coronary circulation would therefore resemble the cerebral (34) in this fundamental respect. This hypothesis which was previously enunciated and investigated by Shipley and Gregg (36), seemed to us to be of sufficient theoretical as well as practical importance to justify further study, and this was the primary purpose of the experiments reported below. More specifically, we desired evidence bearing on the actual metabolic requirements of the heart at the time coronary flow was measured and on the relations among coronary flow, cardiac metabolism, work and efficiency.

Cardiac metabolism has been studied extensively but usually under conditions more or less remote from normal. There is some information about excised heart tissue (see (3)), and considerable about the heart-lung preparation (9) (10) (11) (13) (15) (17) (43) and the isolated heart perfused with blood (1) (18) (19) (20) (21) (30) (43), but we know of only three instances in which data were obtained on preparations with the heart beating *in situ*. Harrison, Friedman and Resnik (14) utilized open and closed chest dogs, measuring coronary blood flow (and thus total oxygen consumption of the heart) by means of an adapted Morawitz cannula inserted into the coronary sinus. It was assumed that coronary sinus outflow represented 60 per cent of the total coronary flow, a percentage that Katz *et al.* (17) have shown is not constant. Wearn (40) has reported cardiac metabolism experiments on the dog with open chest and closed chest with the heart exteriorized. Shipley and Gregg (36) in investigating factors controlling coronary blood flow utilized the open chest dog with blood flow measured by the rotameter.

<sup>1</sup> The expenses of these experiments were defrayed by a grant from the Life Insurance Medical Research Council.

<sup>2</sup> A preliminary report of this work was presented before the Physiological Society of Philadelphia, November 19, 1946. *Am. J. Med. Sc.* **213**: 123, 1947.

<sup>3</sup> National Research Council Fellow in Anesthesiology.

The experience of previous workers suggests that a suitable preparation should fulfill these requirements:

1. The heart should be *in situ* and performing a normal type of work, i.e., blood pressure and cardiac output should be within more normal limits.
2. The heart should be under its own nervous and humoral control.
3. The coronary vessels should be supplied with the animal's own blood at normal body temperature.
4. Measurements of coronary blood flow should be accurate and dependable.

The method used in our previous study of the coronary circulation (8) fulfills these requirements reasonably well. Anesthesia and cannulation of the coronary artery under investigation, a procedure which may interfere with its normal vasomotor innervation, are the chief alterations from normal. Present information indicates that these are minor objections.

**METHODS.** Dogs, unselected as to breed, age or sex and weighing 15 to 22 kgm., were used. They were anesthetized with nembutal sodium (30 mgm. per kilo intravenously with small supplements as required); a few experiments were made during anesthesia by morphine-chloralose, cyclopropane-oxygen, and two with morphine alone following recovery from cyclopropane-oxygen anesthesia. Anesthesia was minimal, a positive corneal reflex usually being present.

1. *Measurement of coronary artery blood flow.* This was done with the bubble flow-meter as in our earlier study (8). In the present experiments flow was measured only in the anterior descending coronary artery because of the choice of source for coronary venous blood.

2. *Collection of coronary venous blood.* Our intention was to estimate cardiac oxygen consumption by multiplying the coronary arteriovenous oxygen difference by the coronary blood flow. The simplest approach was to collect blood from the coronary sinus by means of a catheter introduced through the right auricular appendage, and this was done in 20 experiments before we abandoned it for two reasons. First, we were unable to anchor the catheter so as to prevent obstruction of the coronary veins by the catheter or a withdrawal of the tip of the catheter to a position so close to the ostium as to permit free admixture of blood from the right auricle; a Morawitz cannula was deemed inadvisable because it might have blocked a number of small veins emptying into the sinus just inside the ostium. The second (and more important) reason is that the blood collected from the coronary sinus, under the conditions of these experiments, did not correspond with blood from the coronary veins. This conclusion is implicit in the findings of Katz *et al.* (18) and Moe and Visscher (30) on the isolated heart preparation. Additional evidence obtained in these experiments is as follows:

In order to evaluate the extent to which our method of cannulating a coronary artery interfered with its vasomotor innervation, we attempted to apply to the coronary circulation the nitrous oxide method used by Kety and Schmidt (24) to measure cerebral blood flow. Venous blood samples were collected from the coronary sinus and arterial samples from the afferent limb of the bubble meter while the animal was inhaling 40 per cent  $N_2O$  in 60 per cent  $O_2$ . A representative result is shown in figure 1, which also includes a curve obtained from blood collected from the vein accompanying the anterior descending coronary artery,

one obtained from blood collected from the right auricle, and another calculated for a pure (monophasic) system. It is evident that blood from the coronary vein closely approaches the ideal for a pure system while that from the coronary sinus is intermediate between this and the mixed venous blood. We regard this as strong evidence that blood collected from the coronary sinus may be contaminated with mixed venous blood. We therefore abandoned the coronary sinus and adopted the great cardiac vein as the source of the venous blood for all of the experiments described except those in part 1 of the results. A silver cannula was tied into this vein or into one of its major branches. Blood was allowed to

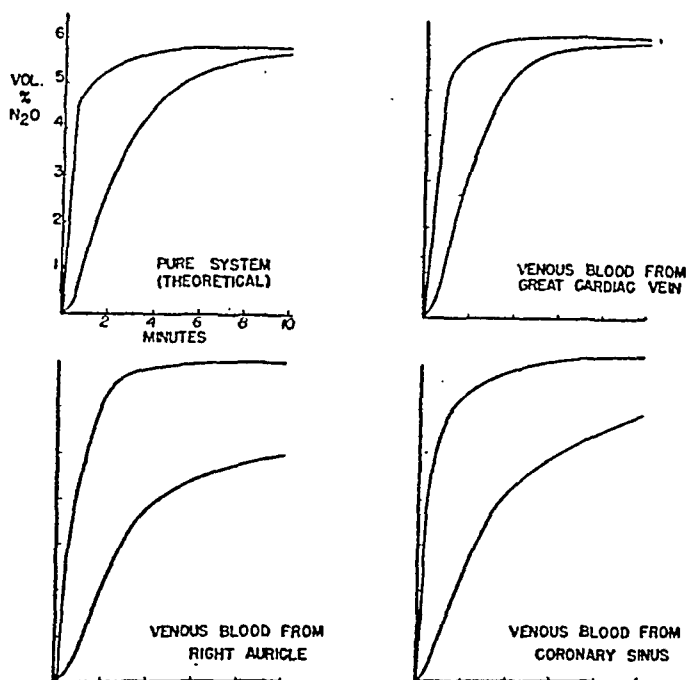


Fig. 1. Nitrous oxide curves with the source of venous blood samples indicated. The pure system curve is an example of one to be expected in the case of a homogeneous flow through a homogeneous tissue. Units and designation of the ordinates and abscissae are the same in all four curves.

flow freely from the collecting system throughout the experiment; when samples were not being collected for analysis the blood was returned via the animal's femoral vein at frequent intervals.

The technic as used in the experiments is shown in figure 2. It is noteworthy that both the coronary arterial and venous distributions pertain predominately to the left ventricular wall.

3. *Collection of other blood samples.* Arterial blood was collected from the afferent limb of the flow-meter. Mixed venous blood (for measurement of cardiac output) was withdrawn from the right ventricle through a small flexible catheter. The proper position of this catheter was confirmed at the end of each experiment.

The collections, which were made into oiled 10 cc. all-glass syringes, were accurately synchronized. Each sample measured about 6 cc. The syringes were immediately capped and immersed in ice-water; the samples were kept in a refrigerator until they were analyzed for their  $O_2$  and  $CO_2$  content by the manometric method of Van Slyke and Neill (38). All analyses were made in duplicate and were checked by additional determinations in case of discrepancy.

4. *Cardiac output.* This was estimated by the direct Fick method based on oxygen, as in our previous study (8). For experiments involving the inhalation of mixtures low in oxygen, total oxygen uptake was estimated from the difference in  $O_2$  content between the inhaled and exhaled volumes of gas (29) (41), the usual method of rebreathing  $O_2$  with the absorption of  $CO_2$  being obviously not applicable. Cardiac rate was obtained from electrocardiographic tracings.

5. *Calculation of left ventricular work.* This was done as a first approximation by the formula  $W = VP + \frac{M\bar{v}^2}{2g}$  which differs from the ideal by the substitution of mean values instead of integrals.

$W$  = Work;  $V$  = Cardiac output in cubic centimeters;  $P$  = Mean arterial blood pressure in millimeters Hg;  $M$  = Mass of blood ejected;  $\bar{v}$  = Mean ejection velocity;  $g$  = Acceleration due to gravity.

6. *Calculation of cardiac efficiency.* This was taken as the ratio between mechanical work and oxygen consumed, both in heat equivalents, and was computed as follows, (28) (41):

Oxygen consumed in heat equivalents =  $O_2$  consumption of the left ventricle  $\times$  caloric value of  $O_2$  at the heart's RQ.

Efficiency = left ventricular work/left ventricular oxygen consumption.

RESULTS. 1. *The "normal" oxygen content of coronary venous blood.* In the early experiments in which blood was collected from the coronary sinus it was apparent from the outset that this blood contained only 4-6 vols. per cent  $O_2$  when right ventricular blood contained 12-16 and arterial blood 19-20 vols. per cent. This was in agreement with the results of other investigators (14) (36) (40). We sought to determine before proceeding further whether the low coronary value was a correct figure or whether it was related to artifacts such as venous stasis induced by our procedures, or the influence of the anesthetic agents.

Venous stasis as a causative factor was excluded by three sets of procedures: 1. Collection of blood from the coronary sinus through a 19 gauge needle thrust directly into it without interference with any coronary veins. 2. Section of a cardiac vein, the escaped blood running down into the pericardial sac where it was collected under oil. 3. Direct inspection of the cardiac vein at the site of insertion of the venous cannula. The samples collected under 1 and 2 still showed the usual low  $O_2$  content; even in 2 the  $O_2$  values were distinctly lower

<sup>4</sup> Mean ejection velocity is computed from the following formula:

$$\bar{v} = \frac{\text{Cardiac output per second}}{\text{Cross sectional area of aortic root}}$$

than in mixed venous blood, and were actually only slightly higher than in coronary vein samples taken in the usual manner. In *S* there was no visible sign of venous distention beyond the cannula, which could be seen through the vein wall.

The effect of anesthesia was investigated by collecting coronary sinus blood samples during three types of anesthesia, viz., nembutal, morphine-chloralose, and cyclopropane-oxygen. Two experiments were completed with morphine alone following insertion of a catheter into the coronary sinus under preliminary cyclopropane-oxygen anesthesia. In the latter experiments arterial and coronary sinus blood samples were taken when the animal moved his head and responded to the spoken voice.

TABLE 1

	NEMBUTAL	MORPHINE- CHLORALOSE	CYCLOPROPANE- O <sub>2</sub>	MORPHINE
No. experiments.....	9	7	4	2
No. trials.....	32	16	4	4
Mean arterial O <sub>2</sub> content vol. per cent.....	17.6	19.2	19.2	16.0
Mean cor. sinus O <sub>2</sub> content vol. per cent.....	5.6	5.1	6.0	3.3
Mean A-V (cor. sinus) O <sub>2</sub> difference vol. per cent.....	12.0	14.1	13.2	12.7

TABLE 2

*The "normal" oxygen consumption of the dog's left ventricle*

		COEFF. VAR. %
Oxygen consumption, cc./100 grams/min.....	8.8	±13.0
Cardiac output, L./min.....	1.3	±20.0
Coronary flow, cc./100 grams/min.....	66.0	±13.0
Mean art. blood pressure, mm.Hg.....	124	±19.0

The results of these four sets of experiments are summarized in table 1. Since there were no marked differences among the four groups we concluded that the low O<sub>2</sub> content of blood collected from the coronary sinus is not solely or largely due to the anesthetic agents used, although we cannot eliminate anesthesia entirely as a contributing factor.

2. *The "normal" oxygen consumption of the left ventricle.* The average findings in 19 technically sound experiments are summarized in table 2. These figures pertain to the control periods.

The value for "normal" cardiac O<sub>2</sub> consumption is considerably higher than the 6.0 cc./100 grams of left ventricle/minute, given in a preliminary report (7). The latter was based on experiments in which coronary sinus blood was used for the calculation whereas the present 8.8 cc. is derived from blood collected

directly from the coronary veins. Previously reported figures in the same range as our present one are 7.8 cc./100 grams heart/minute given by Cohn and Steele (2), and 8.8 cc./100 grams heart/minute reported by Harrison *et al.* (14) in the series of open chest experiments. In the latter connection it is interesting to note that in spite of the unreliability of the coronary sinus blood for representing true coronary venous blood, the average  $O_2$  consumption figure is the same as reported herein although the individual observations indicate great variability. We noted the same variability in the group of our experiments in which the coronary sinus was utilized for obtaining cardiac venous blood.

The high value for cardiac oxygen consumption in our experiments is probably due to some or all of the following: 1. We were dealing with the left ventricle only. 2. The coronary venous blood in these experiments could not have been contaminated by blood from the right auricle. 3. This preparation was closer to normal, including work performed. Although the cardiac outputs of these animals tended to be lower than those regarded by Wiggers (42) as normal for dogs, they were considerably higher than those reported in earlier studies of cardiac metabolism (10) and were within a similar range reported by Harrison *et al.* (14). The blood pressures in our animals also were in the range reported as normal for the anesthetized (42) or unanesthetized (4) (23) dog.

3. *Factors influencing cardiac oxygen consumption.* In the first seven experiments we made no attempt to alter conditions but simply took repeated blood samples, gave occasional infusions of blood and saline, and observed the effects of hemorrhage. In 3 experiments we gave terminal injections of epinephrine and collected a final set of samples while its action was at a maximum. Analysis of the findings in these experiments indicated the following correlations, which were fully borne out by subsequent work:

a. Between cardiac oxygen consumption and coronary blood flow (fig. 3). This is the best correlation of all ( $r = 0.85$ ,  $p < 0.001$ ). Since coronary flow is closely correlated with arterial blood pressure (8) a correspondingly good correlation exists between the latter and cardiac oxygen consumption ( $r = 0.67$ ,  $p < 0.001$ ).

b. Between cardiac oxygen consumption and left ventricular work. This correlation is not as good as the above correlations ( $r = 0.50$ ,  $p < 0.001$ ). Obviously other factors are involved here, the chief one being variations in cardiac efficiency.

c. Between cardiac oxygen consumption and coronary resistance ( $P/F$ ). This relation is poorer than the preceding ones. ( $r = -0.43$ ,  $p < 0.001$ ).

d. Between cardiac oxygen consumption and cardiac output. As might be expected from the fact that the correlation with left ventricular work is relatively poor while that with blood pressure is good, the correlation with cardiac output (which enters equally with blood pressure in the calculation of cardiac work) is the poorest of all ( $r = 0.09$ ).

These findings indicated obscure but important interrelationships among arterial pressure, cardiac output and cardiac efficiency which we next sought to

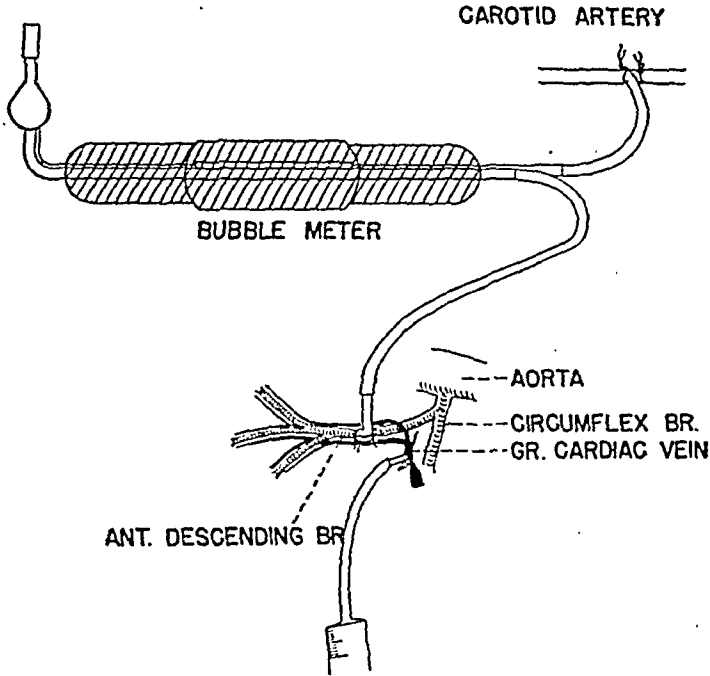


Fig. 2. Schematic representation of the experimental method. *Circumflex Br.* and *Ant. Descending Br.* refer to the branches of the left coronary artery. The syringe shown is connected only during the withdrawal of the coronary venous blood samples.

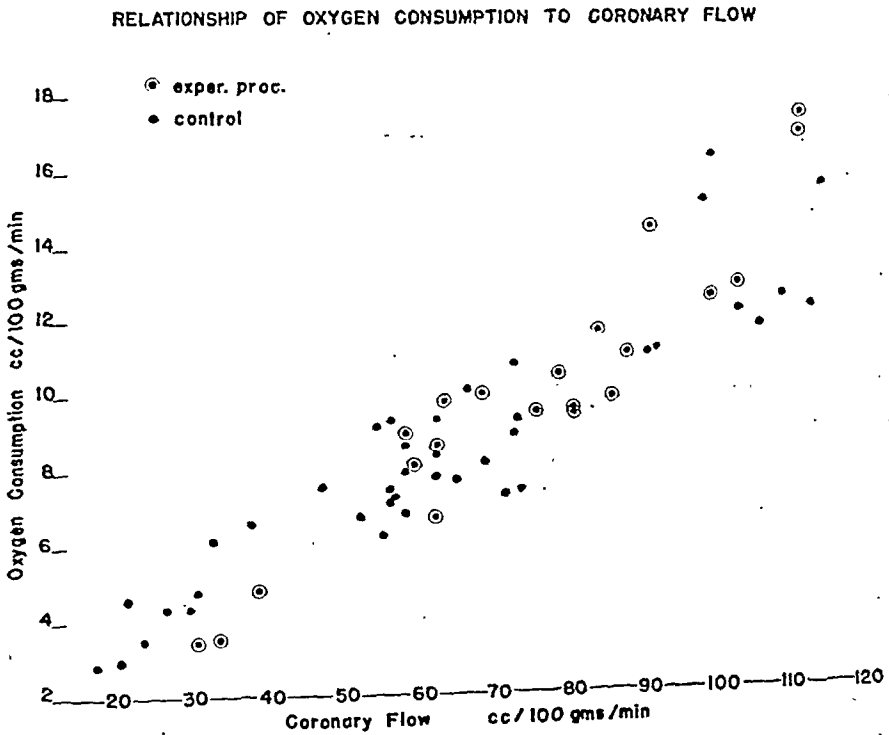


Fig. 3. ● Observations during control periods. ⊙ Observations during experimental procedures. These figures include observations obtained during all experiments reported.

elucidate by studying the effects on cardiac metabolism of changing either the pressure or the output without consensual changes in the other. To raise arterial pressure we resorted to occlusion of the aorta just below the diaphragm and to increase cardiac output we gave an intravenous infusion of fresh donor dog blood and/or gelatine. The experiments and results were as follows:

A. *Effects of primary increase in mean arterial blood pressure.* Four experiments were completed in this group. Through a midline incision a loose ligature was passed around the aorta proximal to the origin of any abdominal branches. The ligature was passed through a glass tube flanged at its lower end. The aorta was compressed against the flanged end by pulling on the ligature. A complete experiment involved at least four sets of readings and blood samples: (1) during the control period; (2) 2-3 minutes and (3) 10 minutes after aortic occlusion was begun; (4) about 20 minutes after release of the occlusion (which was done gradually), when the blood pressure had become stable again. It was also our practice to replace an amount of donor-dog blood equal to that withdrawn in the samples immediately after the samples were obtained.

The results of these experiments, both average and individual, are summarized in figure 4 (A-F). The chosen procedure successfully accomplished the desired purpose, for arterial pressure was elevated consistently although cardiac output was as consistently decreased. Coronary flow was increased as was to be expected from the rise in blood pressure. Another finding was the marked and consistent increase in cardiac oxygen consumption during the period of elevation of arterial pressure. Since cardiac work was decreased at the same time (the decrease in cardiac output being dominant over the increase in arterial pressure in the calculation), cardiac efficiency was also consistently decreased. All of these changes were more or less completely reversed after the normal pressure relations were restored by reopening the abdominal aorta.

B. *The effects of primary increase in cardiac output.* Here also four experiments were completed. To increase cardiac output freshly drawn heparinized dog blood and/or isotonic (4 per cent) gelatin in saline to a total of 300-400 cc. was injected intravenously over a period of fifteen minutes. A complete experiment comprised four sets of readings and blood samples: 1, control period; 2, immediately following completion of the infusion; 3, early (about 10 min.) and 4, late (about 30 min.) after the infusion.

The average and individual results of these experiments are shown in figure 4 (G-L). It should be noted that in 3 of the experiments cardiac output was subnormal at the start while in the fourth it was within our "normal" range. The results, while of the same nature in all, were much less striking in this fourth experiment. Blood pressure, coronary flow, and cardiac oxygen consumption were increased in this series as consistently as in the aortic occlusion experiments. Cardiac output, however, was increased (as intended) and cardiac work was markedly increased because both cardiac output and blood pressure were elevated. Cardiac efficiency was increased here, the work having increased disproportionately with the oxygen consumption.

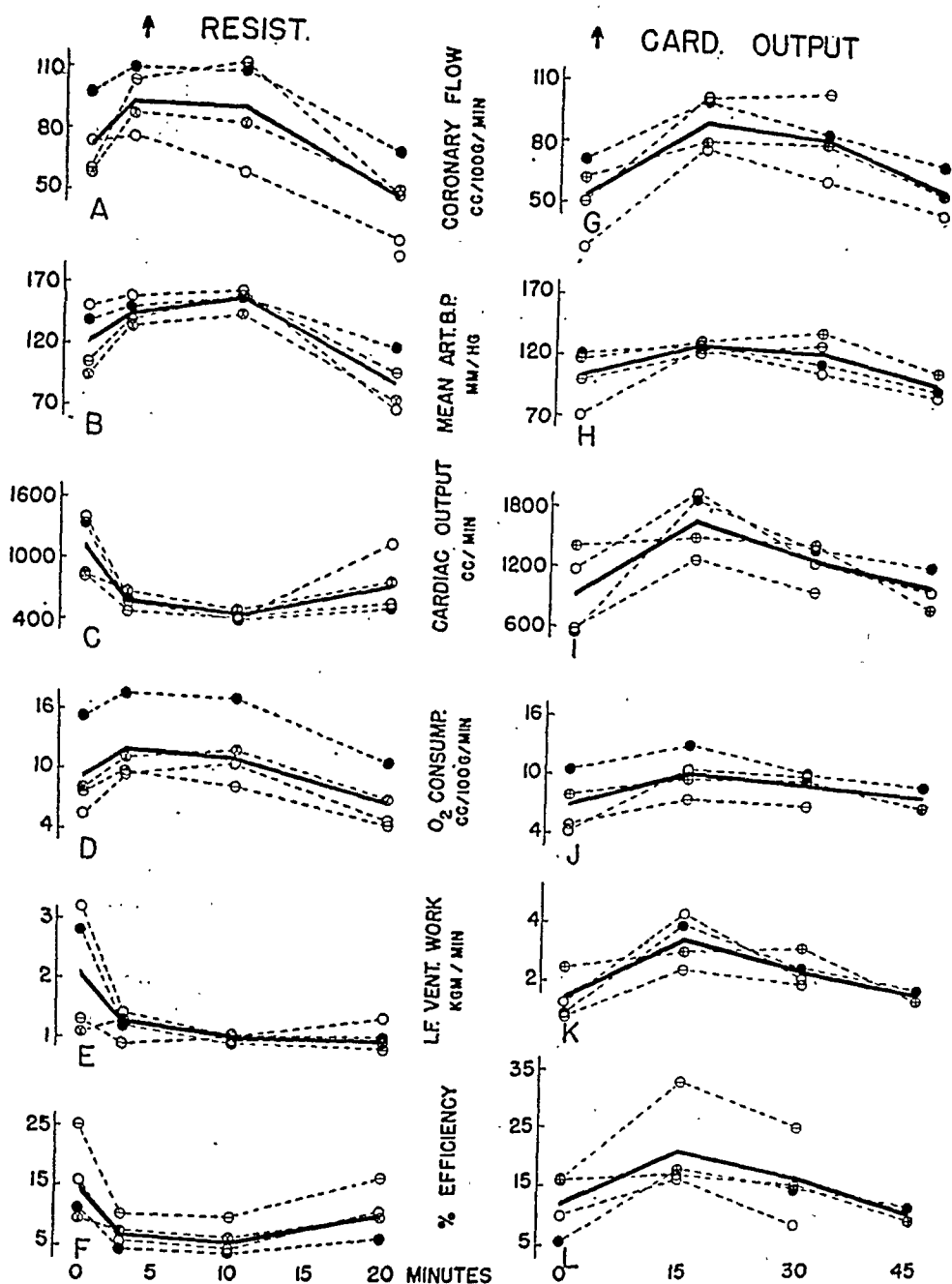


Fig. 4. ↑ *Resist* = experiments in which blood pressure was primarily increased by constriction of the high abdominal aorta. The control observations are indicated at 0 minutes. Duration of constriction was from 0-10 minutes. ↑ *Card. Output* = experiments where the cardiac output was primarily increased by I.V. infusions of blood and/or gelatin. Control observations indicated at 0 minutes. The fluid was administered between 0-15 minutes. ⊗, ⊖, ○, ●, ⊕, indicate experiments 79, 85, 86, 87 and 89 respectively. The averages are shown as heavy lines, the individual observations as dots connected by broken lines.

These findings validate the hypothesis which the present study was intended to test, for coronary flow consistently followed cardiac oxygen consumption under all of the experimental conditions, although it has likewise followed

changes in blood pressure to a lesser degree. As a final test of the thesis that the coronary vessels are in some way regulated by the requirements or products of metabolism we carried out a series of experiments in which cardiac output, cardiac oxygen consumption and coronary flow were measured in dogs subjected to anoxemia produced by inhalation of mixtures low in oxygen.

C. *Effects of primary changes in arterial oxygen content.* Five experiments were satisfactorily completed; a similar number had to be discarded because of technical imperfection, usually in the estimation of total oxygen uptake (hence in cardiac output). The preparation differed from that already described in provision for measuring by two Tissot spirometers the volume of inhaled and exhaled gas (29). The contents of the two spirometers were analyzed for oxygen content at the end of the period of measurement of cardiac output and the volume of oxygen absorbed calculated by the formula:

$$O_2 = \text{Vol. gas inspired} \times O_2 \text{ conc.} - \text{Vol. gas expired} \times O_2 \text{ conc.}$$

The  $O_2$  content of the inhaled gas varied from 100 per cent through 21 (room air), 18, 16, 12, 10 to 8 per cent. The results of the 5 satisfactory experiments are summarized in figure 5.

In general as the arterial oxygen content fell coronary flow increased while blood pressure and cardiac oxygen consumption remained relatively constant. This agrees with the findings of Hilton and Eichholtz (15) and Kiese and Garan (27).

At the same time cardiac output decreased slightly and the small concomitant change in blood pressure was not enough to counterbalance this in the calculation of cardiac work, which decreased somewhat. Since cardiac oxygen consumption remained practically constant while this was occurring, cardiac efficiency fell considerably.

These findings also support the thesis that coronary flow is automatically adjusted to the oxygen requirements of the heart. In this case we have to deal, not with an increased demand, but with a decreased supply. The associated increase in coronary flow is such as to keep the oxygen uptake from falling in the face of a decrease in the oxygen tension at the arterial end of the capillaries. The decrease in coronary arteriovenous oxygen difference (arterial  $O_2$  content falling considerably and coronary venous content only slightly) is a manifestation as well as an approximate measure of this compensation; its effectiveness is indicated by the maintenance of the cardiac oxygen consumption. The calculated decrease in efficiency appears to be referable more to failure of cardiac output to increase (as it characteristically does in man during anoxia (16) (26) (32) (37) (39)) than to failure of the coronary circulation to meet the requirements of the heart muscle.

Some of the findings in the individual experiments deserve comment. In experiments 0-7 and 0-9 there was no great increase in flow, apparently because there was not much change in arterial oxygen content (fig. 5-F). Experiment 0-10 apparently shows the maximal dilating effect of anoxia on coronary blood vessels. When the final blood samples were taken the blood pressure was still rising although cardiac oxygen consumption was almost exactly the same as

in the control period (fig. 5-E). Shortly afterward, however, evidences of cardiac failure appeared: blood pressure began to fall sharply and coronary flow

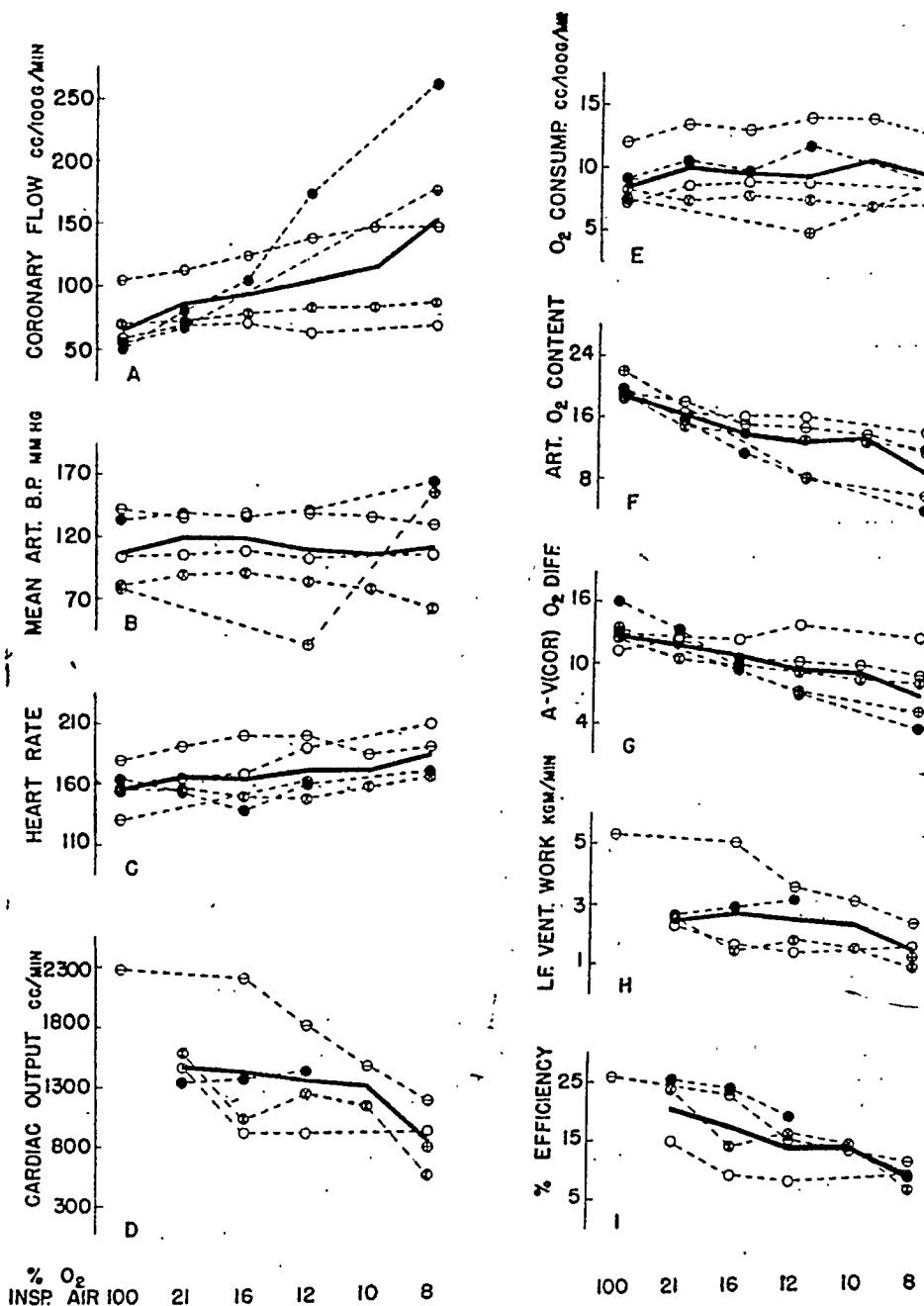


Fig. 5.  $\oplus$ ,  $\otimes$ ,  $\ominus$ ,  $\circ$ ,  $\bullet$ , indicate observations in experiments 0-1, 0-7, 0-8, 0-9 and 0-10 respectively.

Arranged as figure 4 except that  $O_2$  content of inspired air (indicated by  $O_2$  insp. air) appears on horizontal co-ordinate instead of time.

decreased markedly. The arterial oxygen content at this time had fallen to 4 vols. per cent (fig. 5-F). Apparently the oxygen demand by the heart could not be met at this low tension, even with maximal dilatation of the coronary bed.

In examining the data from Greene and Gilbert (12) one would judge it was at a point comparable to this when heart failure occurred.

COMMENT. The original purpose of these experiments, i.e., to examine the thesis that the coronary vessels, like the cerebrals, possess an effective intrinsic control in relation to the metabolic requirements of the tissue, we regard as satisfactorily accomplished. We have been able to show that coronary blood flow, under the varied conditions of these experiments, is adjusted so as to meet primary increases and decreases either in the demand of the heart for oxygen or in the supply in the arterial blood. Our evidence thus is in disagreement with that of Müller, Salomon and Zuelzer (31) who found the rate of oxygen utilization by the heart to have no effect on coronary flow. However our data are in complete agreement with, and add a more precise validation to, the conclusions of Shipley and Gregg (36).

As to the mechanisms involved in these adjustments—whether nervous (an intrinsic nervous system such as that of the intestinal tract) or chemical, and if the latter, the exact identity of the chemical factors—our data do not afford any evidence. From our inability to demonstrate any effective vasomotor control over the coronary circulation under these experimental conditions (8) we infer that chemical factors are the more likely choice and not vagal and accelerator influences as concluded by Sands and DeGraff (33). From our demonstration that anoxemia, excess hydrogen ions, acetylcholine and epinephrine are all capable of dilating the coronaries (8) we conclude that no one chemical agent need be selected as being solely or predominately involved (15) (20); the effect is more likely to be due to the consensual and simultaneous effects of all the demands and products of cardiac metabolism. This agrees entirely with the findings of Shipley and Gregg (36). These conclusions, as well as their supporting evidence, therefore run strikingly parallel to those pertaining to the cerebral circulation (34). The main differences between the two regions, as far as intrinsic vascular control is concerned, are that the coronary vessels of the dog are more sensitive to changes in oxygen tension and hydrogen ion concentration and less sensitive to changes in carbon dioxide tension than the cerebral vessels of man. The importance of the lower sensitivity to  $\text{CO}_2$  must not be regarded too highly in view of the fact that a similar difference exists between the cerebral vessels of intact man and those of the anesthetized, heparinized monkey (6) (25) (35). Whether species differences or artifacts of the experimental conditions are involved cannot be decided at present.

That anoxia should affect the coronary vessels more than the cerebral vessels is perhaps to be expected from the lower oxygen saturation of coronary venous blood. It is interesting to note that coronary blood flow (per 100 grams of left ventricle per minute) in the anesthetized dog is appreciably greater than cerebral blood flow (per 100 grams of brain per minute) in normal man, the figures being 65 and 54 cc. respectively. The corresponding figures for oxygen consumption are 8.8 cc. for the dog's heart and 3.3 cc. for the human brain.<sup>4</sup>

<sup>4</sup> These figures are lower than those appearing in the report cited (25), which were 66 cc. for blood flow and 4.5 cc. for  $\text{O}_2$  consumption per 100 grams of brain per minute. Subse-

Drabkin (5) has pointed out that there may be some causal relationship between the oxygen consumption of the two organs and their cytochrome C content, which is approximately five times as high in cardiac muscle as in the brain.

Apart from the original purpose of these experiments, our procedures enabled us to derive information bearing on the correlations among blood pressure, coronary flow, cardiac output, cardiac work and cardiac efficiency. Perhaps the most important finding is that, under the conditions of these experiments, cardiac efficiency tended to vary directly with cardiac output and inversely with arterial blood pressure when each of these was altered primarily. We did not observe the consistent correlation between cardiac output and coronary flow found by Katz (22); as shown in figures 4 and 5 these two variables frequently moved in opposite directions in our experiments. Our findings in relation to the influence of changes in blood pressure or cardiac output on cardiac efficiency agree in general with those of Gollwitzer-Meier (11), Gremels (13), and Katz (21). All three of these groups however maintained cardiac output relatively constant when blood pressure was increased; the sharp drop in output in our experiments accounts for the more striking decrease in cardiac efficiency.

From our data it appears that one consequence of the normally low oxygen content of coronary venous blood is to throw an inordinate responsibility on the volume of coronary blood flow in meeting an increased demand of the heart muscle for oxygen. In our experiments coronary venous blood under "normal" conditions contained only 4-6 vols. per cent of oxygen and was only 20-35 per cent saturated. Also under these conditions the heart was removing 75 per cent of the total amount of oxygen delivered to it. Obviously there was little room here for removing appreciably larger amounts of oxygen from the blood in response to an increased demand such as might be imposed by higher arterial pressure. There is, however, a compensatory mechanism, viz. the decrease in work (and hence in oxygen demand) associated with circulatory collapse. In our experiments the "normal" oxygen content of arterial, mixed venous and coronary venous blood was roughly 20, 15, and 5 vols. per cent respectively. In circulatory collapse these frequently became 18, 2 and 2 vols. per cent and in several instances coronary venous blood actually contained more oxygen than mixed venous blood collected from the right ventricle. This relation is implicit in the tendency in the present experiments for cardiac efficiency to vary inversely with the blood pressure and directly with cardiac output. When the blood pressure was increased primarily, cardiac output and cardiac work fell while cardiac oxygen consumption increased, with consequent decrease in efficiency (fig. 4); this would imply extravagant utilization of oxygen by the heart muscle. When cardiac output was primarily increased, cardiac work, cardiac oxygen consumption and cardiac efficiency all increased, even though the blood pressure

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quent work has indicated that the solubility factor used in calculating cerebral blood flow by the  $N_2O$  method should be 1.0 instead of 1.3; recalculation with the lower factor gives the results cited above. Reports bearing on these recent findings are to be published in the Proc. Assoc. Amer. Physicians, Am. Jour. Psychiatry and Fed. Proc.

rose concurrently (fig. 4); this implies greater economy of oxygen. When as a result of anoxemia cardiac output fell while blood pressure and oxygen consumption remained unchanged, cardiac efficiency decreased (fig. 5). All of this suggests that a fall in blood pressure without corresponding decrease in cardiac output, such as may occur in spinal anesthesia or circulatory collapse, may not be as ominous as is generally believed. Certainly this particular point deserves further investigation.

Another factor operating in the same direction is the diversion of a larger fraction of the cardiac output into the coronary circulation when cardiac output is reduced by hemorrhage or circulatory collapse. We have already shown (8) that this proportion may increase from the normal 4-5 per cent to 15 per cent or more under such conditions. Thus the oxygen content of coronary venous blood (hence the mean oxygen tension in the coronary capillaries) is reduced less than that of mixed venous blood, not only because the work of the heart is decreased but also because its blood supply is reduced proportionately less than that of the rest of the body. Corresponding observations bearing on the brain are not available; perhaps the impaired consciousness associated with circulatory collapse is an approximate counterpart, but there is at present no basis on which to estimate cerebral work or efficiency.

Thus the heart appears to be safeguarded against dangerous anoxia by three mechanisms, viz. *a*, decreased tonus in the coronary vessels (presumably because of the requirements or products of myocardial metabolism); *b*, diversion of a relatively larger fraction of total cardiac output to the coronary circulation, and *c*, decreased cardiac work, perhaps associated with increased cardiac efficiency consequent to the fall in aortic pressure. The advisability of employing measures intended primarily to raise arterial pressure under such conditions deserves re-examination in the light of these findings.

The procedures employed in this study have shown themselves to be well adapted to a study of the effects of drugs on cardiac oxygen consumption, work and efficiency. Work along these lines is now under way.

#### SUMMARY

1. The development of a method for determining the oxygen metabolism of the left ventricle of an anesthetized spontaneously breathing dog is described and by its use the "normal" oxygen consumption of the left ventricle was found to be 8.8 cc./100 grams/minute.

2. Cardiac oxygen consumption was found to have highly significant correlations with coronary flow, coronary resistance, blood pressure, cardiac work, and no correlation with cardiac output.

3. The data of three groups of experiments are presented in which: 1, arterial blood pressure; 2, cardiac output, and 3, arterial oxygen content was primarily altered, and the effect on cardiac oxygen consumption, cardiac output, cardiac work and efficiency is shown.

4. Coronary blood flow, under the conditions of these experiments, adjusted

itself so as to meet primary changes either in demand of the heart for oxygen or in the supply available in the arterial blood.

5. In these experiments, cardiac efficiency tended to vary directly with cardiac output and inversely with arterial blood pressure.

6. The heart was found to be safeguarded from anoxia in circulatory collapse by three mechanisms *a*, decreased tonus in the coronary vessels; *b*, diversion of a relatively larger fraction of total cardiac output to the coronary circulation; *c*, decreased cardiac work, perhaps associated with increased cardiac efficiency consequent to the fall in arterial blood pressure.

The authors wish to express their gratitude to Dr. Carl F. Schmidt whose keen interest and invaluable suggestions have made this investigation possible. We also wish to acknowledge the advice and assistance of Dr. Seymour S. Kety particularly pertaining to the experiments in which the nitrous oxide method of measuring blood flow was used.

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# THE CONTROL OF BODY-TEMPERATURE IN WHITE RATS

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Rats are useful animals for the study of hypothermia and of hypothermia-producing agents. Yet, little has been done towards establishing the age at which white rats become homeothermic, and few data can be found in the literature on the "normal" variations in body-temperature of these animals. Such data as are available are often presented without adequate descriptions of the manner in which they were obtained.

Efforts to establish these two factors are described in this paper. Details of the techniques employed and the room used for exposure to cold are described elsewhere (Ware, Hill and Schultz, 1947).

*Development of control of body-temperature.* In the only systematic attempt to establish the age at which young rats become homeothermic that we have found in the literature, Brody (1943) placed rats "in a covered container immersed in water at a temperature of 14.7–15.0°C. for fifteen minutes". As nothing is said about ventilation of the container, it can be assumed that it was not ventilated. Without ventilation the decrease in O<sub>2</sub> pressure and the increase in CO<sub>2</sub> pressure in the surrounding atmosphere might be sufficient in 15 minutes, time to modify the control of body-temperature unless the container were large. (Gellhorn and Janus, 1936, and Gellhorn, 1937.) For this reason our experiments on temperature-control were carried out in a large room. The temperature of the room varied between 2 and 4°C., a temperature which we believe is low enough that susceptible animals will show a response before inanition becomes a complicating factor. The temperature of the room in which the rats were kept before the experiment varied from 25 to 27°C.

Rats below 20 days of age were kept with the mothers up to the time of the experiment. These rats as well as the older groups had access to the stock diet (Purina Fox Chow) and water until they were placed in the cold room. In the cold room they were kept in individual wire cages without food or water.

Exposure for 6 hours in the cold room made young rats more susceptible to cold for several days thereafter. For this reason the data reported in this paper are all taken from rats used for the first time.

The curves of figure 1 illustrate the development of the control of body-temperature in normal white rats. Individual curves are given except in figure 1, A, G, H, and J. In the experiments represented by these curves, the data were too closely massed to be presented individually. For this reason, only the average and extreme curves are given. In figure 1, C, D, E, and F, X is a point that would fall on a curve representing the average rate of fall in body-temperature. The numbers in parentheses with X give the average weights of the animals whose temperature curves lie below and above X, respectively. Figure 1,

A represents the fall in body-temperature of 31 rats, 18 days of age when exposed in individual wire cages at  $2-4^{\circ}\text{C}$ . In only one of the 31 rats is there even slight evidence of developing control of body-temperature. At 20 days of age (fig. 1, C) an entirely different picture has developed. A few of the rats have already progressed far toward the control of body-temperature. Even the most rapidly falling curves have less slope than those of the younger rats. The variations in response cannot be ascribed to differences in size. The 14 rats with curves below X had an average body-weight of 27.1 grams; the 14 above had an average body-weight of 29.4 grams. Some of the smallest rats had progressed far in the control of body-temperature. Rats 22 days of age (fig. 1, D) show a smaller proportion that have yet to develop any positive evidence of control of body-temperature, and the average curve (represented by X) has shifted to a higher level. In this group, for the first time, some of the rats show a secondary rise in body-temperature after the primary fall. This response is characteristic of the rats with better control of body-temperature in each of the older groups. The greatest progress in the rats 24 days of age (fig. 1, E) is shown in the partial development of control among the more backward animals. Also, in this group, the average weight of the rats above the median point X is significantly greater than the average weight of those below. The 23 rats 26 days of age (fig. 1, F) have progressed farther along the lines indicated for the preceding group. Only one of the 23 rats 28 days of age (fig. 1, G) shows a continuing fall in body-temperature, and the fall is slight as compared to that of the least well adjusted rats 26 days of age. The average curve for the 23 rats 28 days of age is nearer the high extreme for this group which is a reflection of the fact that only 4 of the 23 at any time showed temperatures below  $36^{\circ}\text{C}$ . The extreme variation in the 18 rats 30 days of age (fig. 1, H) is significantly less than for any younger group. In fact, virtually complete temperature control has been acquired at this age against exposure for 6 hours at  $2-4^{\circ}\text{C}$ . Rats 33 and 36 days of age, for which no curves are presented, did not show any greater degree of control of body-temperature than those 30 days of age.

If the development of the control of body-temperature is influenced by size, it should be possible to bring about the change more quickly by improving the nutritional condition of the rats. This was attempted by reducing litters to 1, 2, 3 and in one case 4, when the rats were two days old. None of the 13 rats from 5 litters so reduced showed any evidence of developing temperature-control at 16 days of age when exposed in individual cages at  $2-4^{\circ}\text{C}$ . One of these rats, the only one kept in a litter of 8, weighed 37 grams, which is more than the average weight of the group 24 days of age with litters of normal size. In several of the older groups, rats weighing less than 37 grams have acquired almost complete control of body-temperature against the stress imposed in these experiments. Size alone, therefore, is not the determining factor. The next group, 18 days of age (fig. 1, B), was composed of 13 rats from 5 reduced litters. As can be seen from the figure, most of the rats of this group have made a significant advance in the control of body-temperature. In 3 of them, control is almost complete. Figure 1, J shows the response of 16 rats from 6 reduced litters 22 days of age. Control in this group is nearly complete.

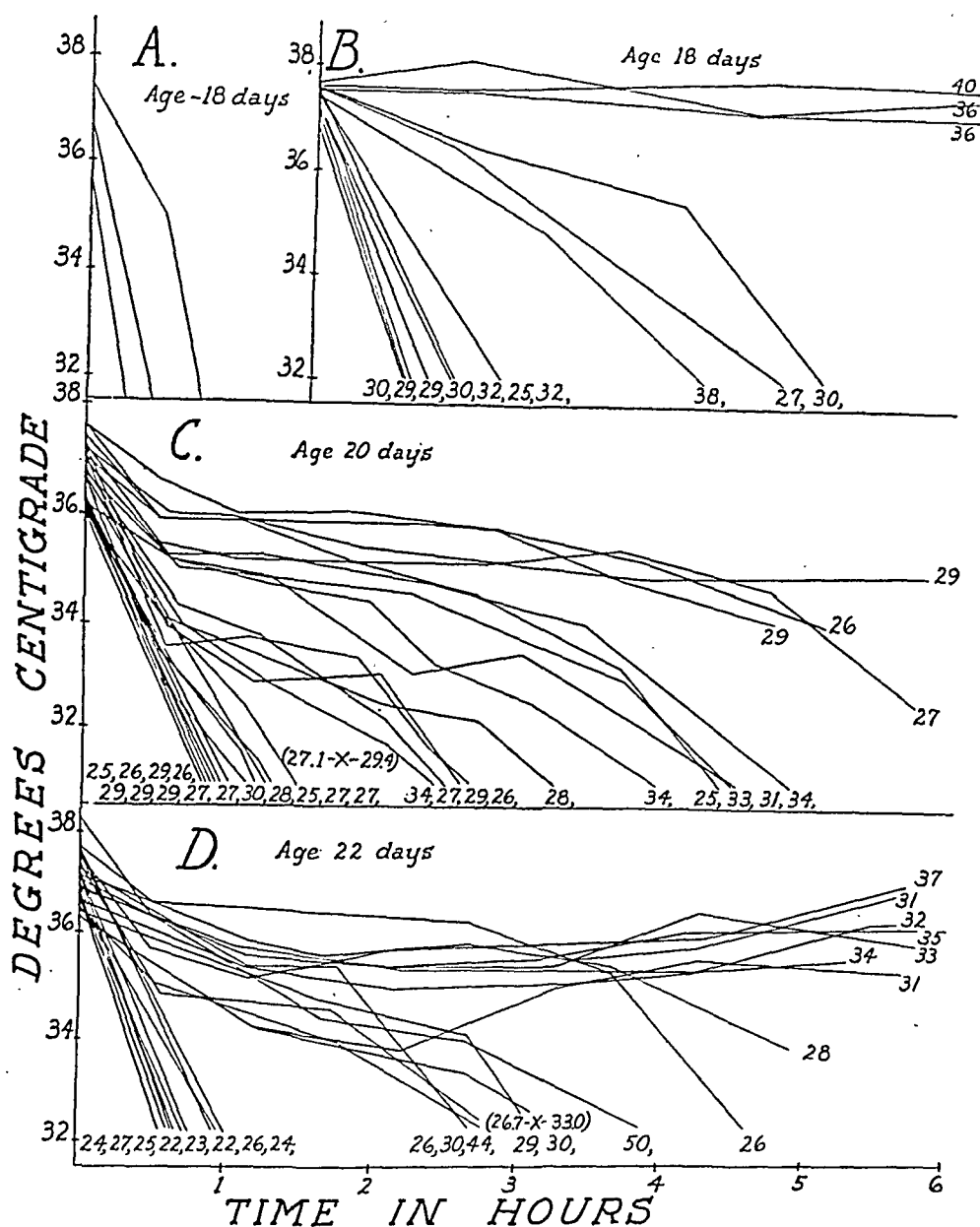


FIG. 1. A-D

Fig. 1. Curves of body-temperature of rats exposed to an environmental temperature of 2-4°C. Numbers on curves, and at bottom in order of rapidity of fall of temperature, indicate weights of individual animals.

A—The average and extreme curves of 31 rats, from 5 litters of normal size. Average weight 26 grams. B—4 reduced litters. Average weight 31.5 grams. C—4 litters of normal size. Average weight 28.3 grams. D—3 litters of normal size. Average weight 29.9 grams. E—4 litters of normal size. Average weight 35.9 grams. F—3 litters of normal size. Average weight 38.9 grams. G—23 rats, from 3 litters of normal size. Average weight 50.2 grams. H—18 rats, from 3 litters of normal size. Average weight 57.7 grams. J—16 rats, from 6 reduced litters. Average weight 42.3 grams.

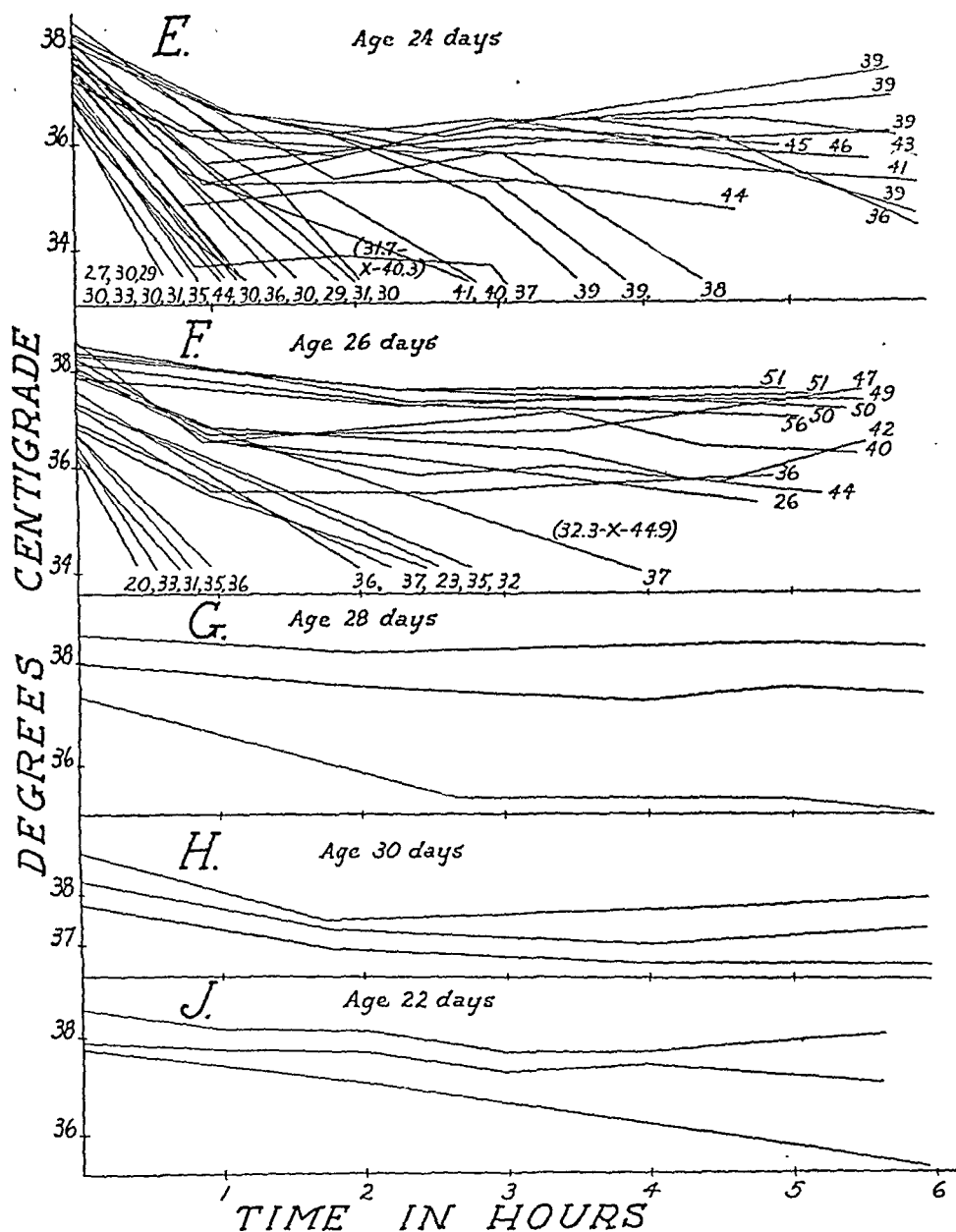


Fig. 1. E-J

Normally, a large group of white rats will exhibit the most rapid development of control of body-temperature against cold over a period of 12 days from the 18th to the 30th day of life. The change in individual rats may be of considerably shorter duration but will fall within this 12 day period. There is also variation between litters. All of the curves for one litter may fall near the top of the range characteristic of its age whereas the curves for another litter may all fall near the bottom. This is particularly true of litters 22 and 24 days of age. In general the rats most susceptible to cold have the lowest initial temperatures.

The earliest evidence of developing control of body-temperature on exposure to cold is a slower loss of heat after a rapid initial fall during the first 30 minutes. In the second stage, the rapid initial fall is followed by a plateau, which begins somewhere between body-temperatures of 33° and 36°C. The plateau which may be of any length up to 4 hours is followed by a second fall. The third stage is like the second except that a rise occurs after the plateau. The fourth and final phase is a narrowing of the extreme range of variation with nearly flat curves as is seen for rats 30 days of age (fig. 1, H).

Rats between the ages of 30 and 60 days maintain normal body-temperature on simple exposure in the cold room, but the control is still easily upset by mild stresses such as taking colonic temperatures at frequent intervals with a cold thermometer. Between the ages of 60 and 300 days, the temperatures of rats can be made to fall in the cold room by use of severe stresses such as keeping the hair wet or reducing the available oxygen, but in this age group, the body-temperature can not be made to fall at environmental temperatures of 2-4°C. by imposing the milder stresses that are effective with the younger group. In rats of this age group, 60-300 days, there is a marked tendency to adjust to body-temperatures between 37° and 38°C. on exposure to 2-4°C. Those having higher initial body-temperatures show a drop during the first hour and those having lower initial body-temperatures show a rise. Consequently, young adult rats will usually show a more narrow range of variation in body-temperature when exposed at 2-4°C. than will the same rats at room temperature. In old rats, the control of body-temperature deteriorates progressively.

*"Normal" variations in the body-temperature of white rats.* Collection of the data presented in this section has been incidental to other research problems over a period of about eight years, but the same standardized procedure was used throughout. Unless otherwise stated, all animals had been in individual wire cages for at least one hour before the temperatures were taken. The environmental temperature varied between 25° and 27°C.

In figure 2, the mean body-temperatures of rats are shown at 2 day intervals between the ages of 18 and 30 days. The numerals at each point represent the number of animals used in arriving at the mean values. When several rats of this age group are allowed to remain together in one cage, so that they can pile up, the mean body-temperature will be distinctly higher than the values shown in figure 1, usually above 38°C. at environmental temperatures above 25°C. The mean body-temperature of rats kept in individual wire cages increases fairly rapidly from 36.5°C. at 18 days of age to 38.2°C. at 30 days. The latter temperature is not materially different from the mean body-temperature of the next older age group.

Figure 3 shows two frequency histograms, one of the body-temperatures of 1181 rats over 60 days of age (solid line) and one of the body-temperatures of 355 rats with ages from 31 to 60 days (broken line). Only 90 of the animals of the adult group are in the age range of 300 to 760 days, but because the mean body-temperature and the distribution are almost the same as for the younger adult rats, the data are included in the same histogram. The mean body-

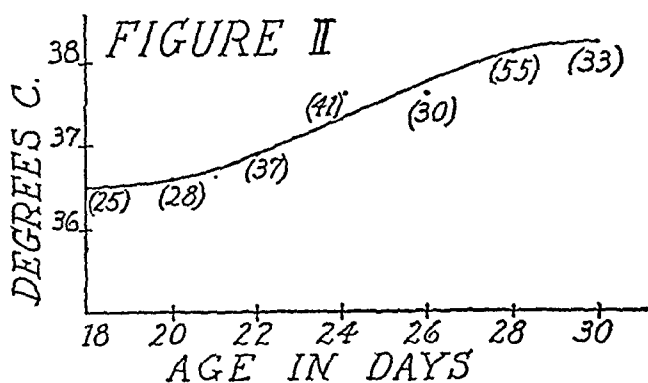


Fig. 2. Progressive increase in mean body-temperatures of rats from 18 to 30 days of age. Numerals on the curve represent the number of rats used in arriving at the mean for each point.

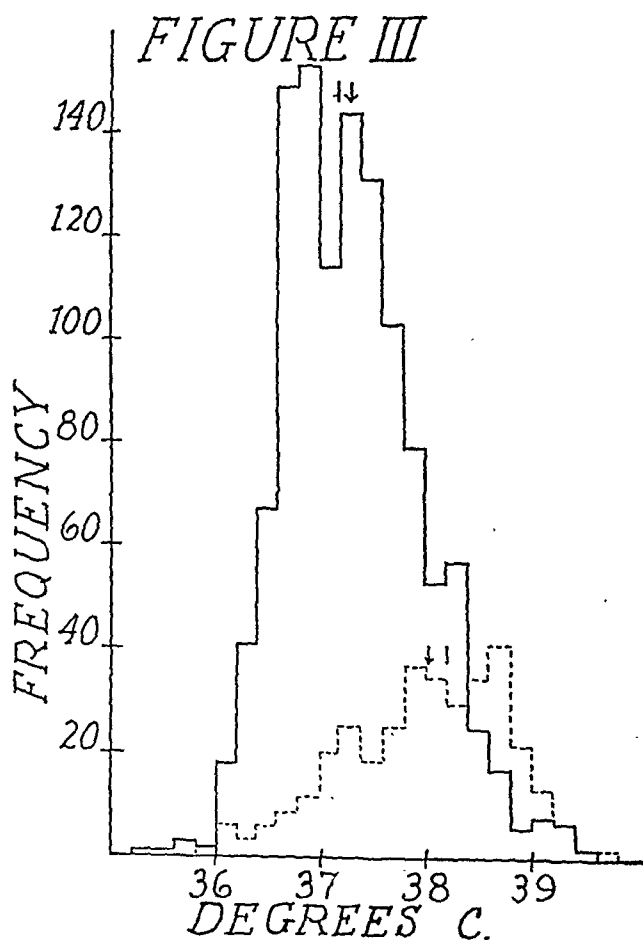


Fig. 3. Frequency histogram of the body-temperatures of 1181 rats from 61 to 760 days of age, solid line; and of 355 rats from 31 to 60 days of age, broken line.  
 ↓ mean body temperature.  
 | median body temperature.

temperature of the adult group (60 to 760 days of age) is  $37.27^{\circ}\text{C}$ . and the standard deviation from the mean is  $\pm 1.38^{\circ}\text{C}$ . The median is at  $37.2^{\circ}\text{C}$ . The mean of the younger group (31 to 60 days of age) is  $38.03^{\circ}\text{C}$ . and the standard deviation from the mean  $\pm 1.02^{\circ}\text{C}$ . The median is at  $38.2^{\circ}\text{C}$ .

The rats of 31 to 60 days of age are about equally distributed between males and females. The mean body-temperature of the females is  $0.3^{\circ}\text{C}$ . higher than that of the males. The rats of figure 3 over 60 days of age are also about equally distributed between males and females, but no significant sex difference in body-temperature is shown in this group.

#### SUMMARY

From the point of view of the control of body-temperature, the life span of the rat may be divided into 5 periods:

1, birth to 18 days, when there is little, if any, resistance to exposure to cold; 2, 18 to 30 days, the period of rapid development of control of body-temperature against cold; 3, 31 to 60 days, a period of slow improvement in temperature control; 4, 61 to 300 days, the period of maximum resistance to cold, and 5, 300 days to death, a period of slow deterioration in the cold resisting mechanism.

The beginning of the 5th is less sharply defined than that of the earlier periods.

With respect to "normal" variations in body-temperature, the life span of the rat can be similarly divided except that the 4th and 5th periods are not differentiated.

Body-weight does not seem to be a factor in initiating the development of control of body-temperature, though once it is initiated, the heavy rat is likely to show a slower temperature fall in cold environments than smaller animals of the same age.

The experiments with rats of reduced litters show that better nutrition produces an earlier development of control of body-temperature, but that this effect also fails to show correlation with increased weight.

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# THE EFFECT OF INTERFERENCE WITH RESPIRATION ON THE CONTROL OF BODY-TEMPERATURE IN WHITE RATS AND NEW ZEALAND RABBITS

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Normal adult rabbits and rats do not experience a fall in body-temperature on simple exposure to low environmental temperatures but can be made to do so by several procedures that do not involve surgery or the administration of drugs. Ariel, Bishop and Warren (1943) produced subnormal temperatures in rabbits by wetting the skin while exposing the animals to cold environments. Gellhorn and Janus (1936) and Gellhorn (1937) produced hypothermia in rats by exposing them to cold in atmospheres with decreased  $O_2$  pressure. Hamilton (1937a, b, c, d) has reported falls in body-temperature in rats caused by "immobilizing" the animals and exposing them to low environmental temperatures.

**METHODS.** During the past seven years, for various purposes, we have produced hypothermia by interference with the respiration and exposure to cold in more than one thousand white rats. In this paper we are reporting some characteristics of the time-temperature curve, certain factors that alter the curve, and some observations made on the animals during hypothermia. Experiments are included in which otherwise similarly treated rats were subjected to room temperature and higher temperatures rather than to a cold environment.

At first we attempted to follow the technique of Hamilton (1937b) for producing hypothermia in rats, which he describes as immobilization in a wire cage, closely fitting but sufficiently loose that the rat can crawl into it unaided. The rats in the cages were exposed in the cold room at  $2^{\circ}$ – $4^{\circ}$ C. However, although many of the animals remained practically motionless, hypothermia could not be produced in healthy adult rats by this technique unless the cages were tight enough to impede breathing. Accordingly, sufficient restraint for this purpose was used in subsequent experiments. We found it convenient to wrap the thorax and abdomen of the animals with strips of adhesive tape as a means of restraint and most of our experiments on hypothermia in adult white rats were carried out using this procedure. This method of restraint reduced not only the amplitude but the frequency of respiration. When animals were exposed without wrapping, they were kept individually in ordinary wire cages. Either Anschutz thermometers with  $\frac{1}{2}$  degree intervals or clinical thermometers specially made for low temperature recording were found amply sensitive for measuring temperature changes of the order recorded in these experiments. In order to obtain reproducible body-temperatures in rats, care must always be observed that high colonic temperatures are taken and that the thermometer at the time of

insertion is itself only a few degrees from the temperature of the experimental animal. The latter condition was achieved by keeping the thermometers immersed in water of appropriate temperature. Further details of the method are given elsewhere (Hill, Ware and Schultz, 1943).

Non-fasted, New Zealand white rabbits were used for determinations of  $O_2$  and  $CO_2$  tensions in the blood during hypothermia. The animals were bound in a manner similar to that used with rats and exposed to an environmental temperature of  $2^\circ$ – $6^\circ C$ . When treated in this way, rabbits show a drop in body-temperature but less profound than that found in rats under like conditions. Colonic temperatures were taken at a depth of 11 cm. Blood was taken anaerobically from the ear veins with a platinum needle without the use of heat and was kept under oil until analyzed. High potency heparin was used as the anticoagulant. The  $CO_2$  and  $O_2$  contents of the blood were determined promptly by the method of Van Slyke and Neill (1924), using 0.2 cc. samples. Determinations of specific gravity were made on the plasma using the falling drop method of Kagan (1938).

The dimensions of the cold room in which the animals were exposed are 4 x 12 x 7 feet. The relative humidity was 75 per cent with little variation on different experimental days. When the rats were exposed at elevated temperatures, a well-ventilated warm room was used, the dimensions of which are 6 x 7 x 7 feet. The relative humidity of the warm room was 23 per cent on the average with unimportant variations. Temperatures were taken in both cases without removing the rats from the room in which they were exposed.

EXPERIMENTS ON RATS. (a) *Characteristics of the temperature curve.* When adult white rats are wrapped and exposed in the cold room, any rate of fall in body-temperature may be achieved, from no fall at all to a rate somewhat faster than that of dead animals exposed in the same way. The rate depends on the tightness of the binding, and therefore, on the extent to which respiration is impeded. Because the slope of the curve depends on the tightness of the binding and because we are unable to give more quantitative expression to the binding-factor than to call it "loose", "medium" or "tight", no attempt is made to average the data of some of our experiments. Instead, from data on individual rats, we are presenting curves each of which is representative of a considerable number of experiments. Figure 1, *A* shows curves representative of loose (*D*), medium (*F*) and tight (*E*) wrapping. The rate of fall in body-temperature in tightly wrapped live animals is also compared with the rate of fall in the same animals after death. The dead animal was warmed in the incubator and the rate of fall in body-temperature was measured a second time in the cold room (*G*).

During the fall in body-temperature in a bound rat, the shape of the temperature curve can be changed almost at will by either loosening or tightening the adhesive strips (fig. 1, *B*). When the strips on a loosely-bound rat are tightened at any time during the fall in body-temperature (*M*), the rate of fall is accelerated. Conversely, when the strips on a tightly-bound rat are loosened (*N*), the rate of fall is diminished.

Frequently rats will remain quiet during the first 4 to 8 degrees of fall in body-

temperature, and then will struggle more or less violently for some minutes. When struggling begins, in the majority of cases, the rate of fall in body-temperature is momentarily retarded. But the decreased rate is of brief duration and is followed by a second more rapid drop which often begins before the struggling ceases. Figure 1, C presents two illustrative cases, one in which the fall in temperature was briefly retarded when struggling began and a second in which, if there was any retardation of the fall in temperature, it was so brief that it was missed. In neither case was any alteration made in the binding of the animal.

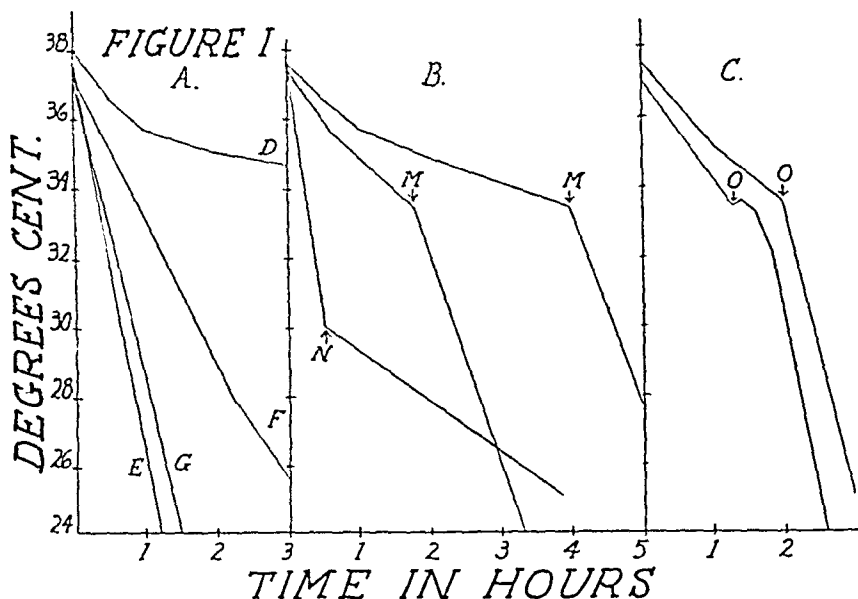


Fig. 1. Exposure of rats in a cold environment (2-4°C.)

A. Rate of fall of body-temperature of loosely bound rats (D), tightly bound rats (E), medium tightly bound rats (F), and dead rats (G).

B. Effect of tightening (M) and of loosening (N) the binding of rats on the rate of fall of body-temperature.

C. Effect of struggling (O) on the rate of fall of body-temperature of bound rats.

(b) *Body temperatures from which rats of different ages will recover at environmental temperatures of 2°-4°C.* Three groups of rats were used: One of rats 45-50 days old; one, 74-120 days old; and one, 20-26 months old. The rats were bound and exposed in the cold room as before. When the colonic temperatures reached the desired level, the animals were unbound and left in individual wire cages in the cold room at a temperature of 2°-4°C. The results of these experiments are shown in figure 2. Differences in response within each group could not be correlated with age. But it is evident that there are significant differences between the groups. The rats of the young adult group (fig. 2, B, 74-120 days old) were more able to recover normal body-temperatures under these conditions than those of either the younger or older groups. All of the 21 animals of this group that were unbound before the colonic temperatures reached 23°C. recovered. In the youngest group (fig. 2, A, 45-50 days old)

only one rat recovered. This rat was unbound when its body-temperature reached  $26.5^{\circ}\text{C}$ . Eleven of this group were unbound when their body-temperatures were still above  $23^{\circ}\text{C}$ . In the oldest group (fig. 2, C, 20-26 months) only three recovered, although nine were unbound at body-temperatures above  $23^{\circ}\text{C}$ .

(c) *Exposure of bound rats at different environmental temperatures.* Figure 3 shows the effects on body-temperature of exposing bound rats (70 days old) at different environmental temperatures. The curves *C* and *C'* indicate that  $31^{\circ}\text{C}$ . is very close to the neutral environmental temperature for these experiments. Exposed at this temperature, rats, whether wrapped or unwrapped, show no greater variations in body-temperature than would be expected at the

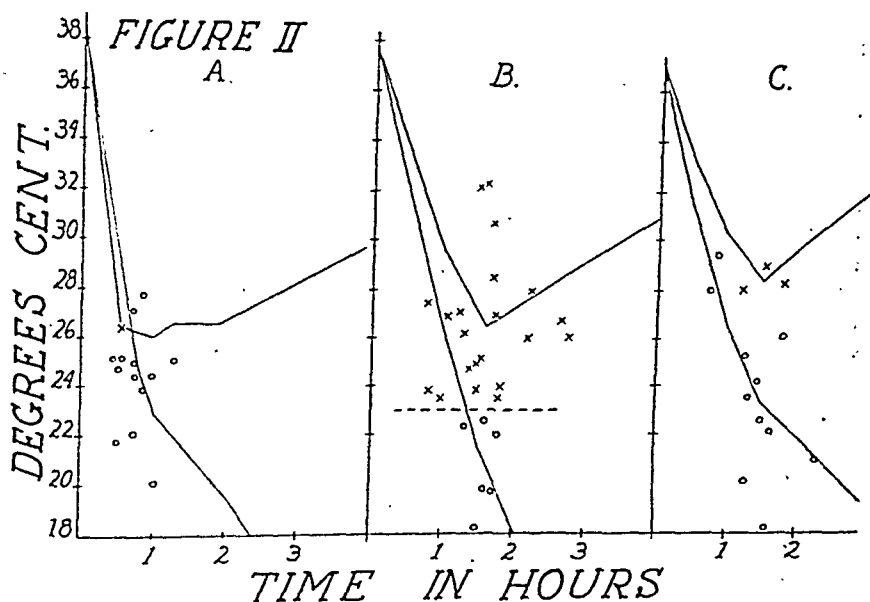


Fig. 2. The recovery of rats from low body-temperatures while kept at an environmental temperature of  $2-4^{\circ}\text{C}$ . The time-temperature position of unbinding each rat that recovered is marked with an X, and each rat that failed to recover with an O.

A: 45-50 days of age. B: 74-120 days of age. C: 20-26 months of age.

usual room temperature with no treatment. At an environmental temperature only  $3^{\circ}\text{C}$ . lower ( $28^{\circ}\text{C}$ .) (*D* and *D'*), unwrapped rats show no change in body-temperature but wrapped rats show a significant drop. At an environmental temperature of  $34^{\circ}\text{C}$ .,  $3^{\circ}\text{C}$ . above the neutral temperature, (*B* and *B'*), both the wrapped and the unwrapped rats show a significant rise in body-temperature, although the rise in the wrapped rats is considerably delayed. When exposed to an environmental temperature of  $40^{\circ}\text{C}$ . (*A* and *A'*), the body-temperature of both wrapped and unwrapped rats rises promptly and to about the same degree. All of the rats exposed at  $40^{\circ}\text{C}$ ., whether wrapped or unwrapped, tolerated the treatment very poorly.

EXPERIMENTS ON RABBITS. Gellhorn and Janus (1936) and Gellhorn (1937) using rats, mice, and guinea pigs as experimental animals, found that decreasing

the  $O_2$ -pressure in the atmosphere without other treatment produces a fall in body-temperature. If, at the same time, the  $CO_2$  in the atmosphere is increased to 3 per cent, the fall in body-temperature is greater and the animals remain in better condition. Our method of binding rats to induce hypothermia reduces both the amplitude and the frequency of respiration. It seemed possible that these conditions might produce an increased  $CO_2$  pressure and a decreased  $O_2$  pressure in the tissues. To test this possibility, rabbits were used as the experimental animals because of the greater ease of obtaining adequate samples of blood.

Twenty-eight experiments were completed on 12 rabbits. After preliminary blood samples were withdrawn for analysis, the animals were bound and placed

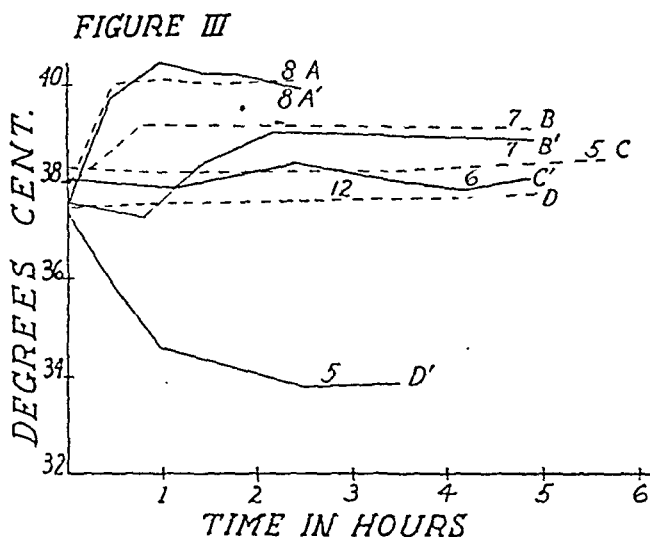
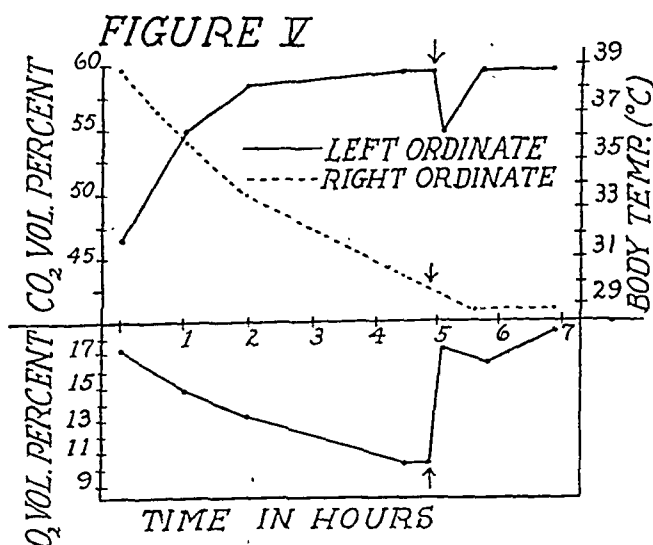
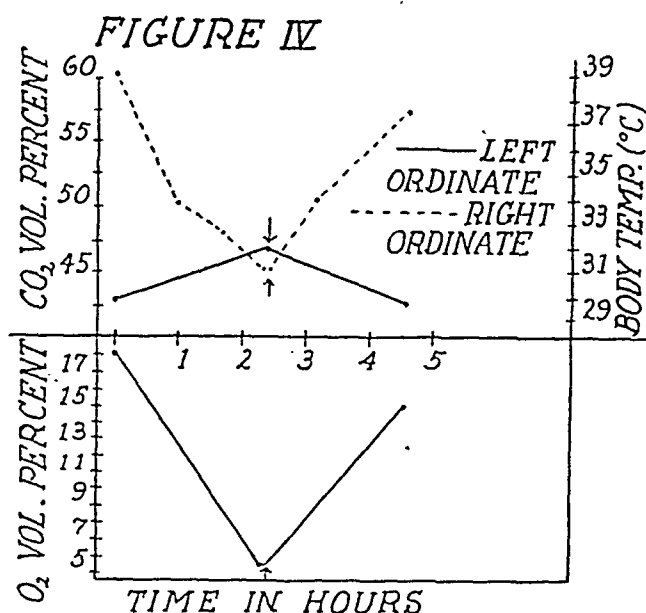


Fig. 3. Effect on body-temperature of exposing bound rats (70 days old) at different environmental temperatures. Solid lines represent bound rats and broken lines represent unbound rats. Environmental temperatures were, A, A' 40°C., B, B' 34°C., C, C' 31°C., and D, D' 28°C.

in the cold room and, at intervals thereafter, further blood samples were taken and the  $O_2$  and  $CO_2$  contents compared with the fall in body-temperature.

Figures 4 and 5 show the results of two individual experiments which are representative of the type and extent of individual variation. In figure 6 the data of 25 of the 28 experiments are combined in average curves. Two are omitted because the initial blood was lost and one because of an extremely high initial  $CO_2$  content that was not considered to be normal. Eight points are shown on the curve. The numbers in parentheses on the curves indicate the number of determinations represented by each point. Blood was very difficult to obtain from animals with body-temperatures as low as 30°C. This operation was successful in only two experiments with body-temperatures at 29°C. No point is given on the curve for  $O_2$  content at this body-temperature because, of the two experiments completed, one showed 1.2 vol. per cent and the other 12.4 vol. per cent decrease. The latter value was obtained from an animal

that had a high initial  $O_2$  content, which continued to fall without showing the usual secondary rise at low temperatures.



Figs. 4 and 5. Changes in the body-temperature and the  $O_2$  and  $CO_2$  contents of venous blood in rabbits on binding the thorax and abdomen and exposing to an environmental temperature of 2-4°C. The arrows mark the time at which the rabbit was unbound.

All the animals showed a pronounced fall in  $O_2$  content of the blood that was closely parallel to the fall in body-temperature. In only one of the 28 experiments did the  $CO_2$  content fail to rise and this was in a case with an exceptionally high initial value for  $CO_2$  (62.6 vol. per cent). However, the  $CO_2$  content of the blood in these experiments was more variable than the  $O_2$  content and its

rise was not always coincident with the fall in body-temperature. Figure 4 presents a picture typical of the results with a number of the rabbits. In this group, the  $O_2$  content of the blood and the body-temperature showed the most rapid decline. But the  $CO_2$  content of the blood in this group showed the least rise. On being unbound, but kept in the cold room, these animals recovered more rapidly than the others.

Figure 5 presents a picture that is typical of the rabbits in which there was a slow fall in  $O_2$  content of the blood and a slow fall in body-temperature. In this group, the rise in  $CO_2$  content was rapid, and to a comparatively high level. When the rabbits were unbound but kept in the cold room, the  $O_2$  content of the blood returned to normal with great rapidity but the body-temperature

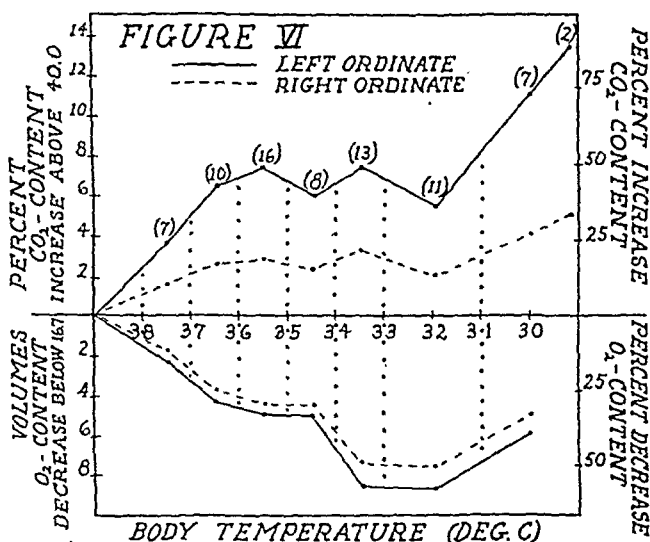


Fig. 6. The changes in  $O_2$  and  $CO_2$  contents of venous blood plotted against the fall in body-temperature. The initial  $CO_2$  content and  $O_2$  content were 40 volumes percent and 16.8 volumes percent, respectively. The values are the means of 25 experiments on 12 rabbits. The numerals on the curves represent the number of bloods analyzed at body-temperatures within the respective dotted lines.

rose very slowly. Coincident with the slow rise in body-temperature there was a retarded return of  $CO_2$  content to the initial level.

In figure 6 the average curves of  $O_2$  and  $CO_2$  content of the blood are plotted against the fall in body-temperature. The  $O_2$  content of venous blood dropped progressively with body-temperature until the body-temperature was about  $33^\circ C$ . At this point, the average  $O_2$  content was 48 per cent of the initial value. The  $CO_2$  content of the blood rose rapidly at the beginning of hypothermia and then leveled off. The  $O_2$  content rose somewhat and the  $CO_2$  content rose sharply as the body-temperature fell below  $32^\circ C$ .

Two of the rabbits were kept at room temperature for  $1\frac{1}{2}$  hours after binding. There was a fall in  $O_2$  content and a rise in  $CO_2$  content of the blood in these animals, but, as long as they remained at room temperature, the body-temperature

did not change significantly. On placing these animals in the cold room, the fall in body-temperature was exceptionally rapid.

Plasma specific gravity and hematocrit determinations were carried out on 4 of the rabbits. These indicated at a body-temperature of 34°C. an average concentration of blood of 6.9 per cent. These changes in concentration would make a small negative correction in the CO<sub>2</sub> content as shown in the figures but would mean that the true fall in O<sub>2</sub> content is correspondingly greater than is shown.

**DISCUSSION.** The curve of body-temperature fall of wrapped rats when exposed to low environmental temperatures is logarithmic. Deviations from the logarithmic curve are due to lack of constancy in conditions, as, for instance, in the tightness of wrapping and in the activity of the animals. The logarithmic character of the curve with tightly wrapped rats (fig. 1, A) is not immediately apparent because of the rapidity of fall in temperature and the limited survival-time of the animals. The finding that tightly wrapped rats suffer a more rapid loss of body-temperature in the cold room than the same animals after death may be explained on the basis of distribution of heat to the surface of the living animal by the blood stream.

Hamilton (1937c) states that, "The rodent has been found to have an exceedingly rapid heat loss in a moderately cool environment (35°F.) without the use of anesthesia or other means of destroying body heat regulation . . .," and again (1937d) "... a cold environment suffices for temperature reduction in small animals." In our experiments normal adult rats not only failed to show falls in body-temperature on simple exposure to an environmental temperature of 4°C., but in many cases were able to recover from markedly subnormal body-temperatures while kept at this cold environment (fig. 2). A possible explanation for the difference between Hamilton's results and ours lies in the following statement of Hamilton (1937a) describing his rats in the hypothermic condition. "The hair stands on end porcupine-fashion and is covered by fleas which have crawled away from the cold skin." The animals used in the experiments we are reporting were free from fleas. However, on one occasion, we found one group of rats infested with fleas. On simple exposure in the cold room at 4°C., these rats experienced a fall in body-temperature of 2° to 8°C. in from 4 to 6 hours. A group of rats that had acquired "snuffles" also failed to maintain normal body-temperatures in the cold room. The question arises as to whether flea infestation may not be a pathological condition of sufficient severity to decrease the resistance of rats to change in body-temperature.

In general, we are in agreement with the descriptions of the reactions of rats in a state of hypothermia as recorded by other investigators. Hamilton (1937a) reports that, "The lowest level of body-temperature from which any animal recovered was 54°F." The age of the animals is not given. In our experiments, the lowest colonic temperature from which a rat recovered varied with age. Newborn rats, when returned to room temperature, recovered from body-temperatures as low as 5°C., and rats 30, 60, 80, and 600 days of age from body-temperatures as low as 8°, 11°, 15°, and 15°C., respectively. Hamilton cooled his rats in "an ordinary ice-box", and observed them through a glass

door. He concluded that "shivering is not a marked phenomenon in the rat." Our rats were cooled in a cold room. The observer was in the room with the rats and could touch them at any time. Shivering was not strong enough to be seen readily but could be felt at all times in nearly all of the animals while they were in the cold room. It began soon after the body-temperature fell below  $37^{\circ}\text{C}$ . and continued in most animals until their temperatures reached  $20^{\circ}\text{C}$ . Shivering continued during the rise in body-temperature if the animals were kept in the cold room but was observed infrequently when the animals were removed to a warm environment.

Hamilton (1937a) states that rats in the hypothermic state when stimulated "... initiate biting movements, turning the head in the direction of the stimulus and opening the mouth, but ... desisting before completion of the action." Most of our rats reacted in similar fashion during hypothermia of short duration. However, in some unpublished experiments, we have seen rats develop a very belligerent attitude toward each other after several days of hypothermia. Two adult male rats with body-temperatures of  $23^{\circ}$  and  $24^{\circ}\text{C}$ ., respectively, on the seventh day of continuous hypothermia, inflicted considerable damage on each other. All movements, both of attack and defense, were very slow, but were completed.

The experiments reported in figure 3, in which the response at different environmental temperatures is compared, suggest that regulation of the  $\text{O}_2$  and  $\text{CO}_2$  levels in the tissues is essential to the maintenance of body-temperature even at environmental temperatures as high as  $28^{\circ}\text{C}$ . This regulation may operate as a protection against abnormally high environmental temperatures (fig. 3, curves *B* and *B'*) but less effectively.

In most of the experiments on rabbits the decrease in the  $\text{O}_2$  content of the blood on exposing the bound animals to a low environmental temperature is so closely parallel to the fall in body-temperature that a cause and effect relationship between the two phenomena seems probable. This is particularly noticeable in some animals not shown individually in the figures, in which light initial binding produced small falls in  $\text{O}_2$  content and, at the same time, only small decreases in the body-temperature. On tightening the binding in these animals, both the fall in  $\text{O}_2$  content of the blood and the decrease in body-temperature were accelerated promptly. That the fall in  $\text{O}_2$  content (or perhaps more strictly the decrease in the rate of oxidative metabolism) is the cause, and the fall in body-temperature is the effect, is indicated by the experiments in which rabbits were kept at room temperature for  $1\frac{1}{2}$  hours after binding. In these experiments, the  $\text{O}_2$  content of the blood fell immediately after binding and the  $\text{CO}_2$  content rose, but the body-temperature remained within normal limits until the animals were exposed to the temperature of the cold room. The fall in body-temperature on exposure to cold was then exceptionally rapid in these animals that have an already depleted  $\text{O}_2$  content of the blood.

From the results of experiments on rabbits, it seems probable that, in our experiments on rats, changes in the degree of restraint (fig. 1, *B*) altered the amount of  $\text{O}_2$  available to the tissues and the rate at which  $\text{CO}_2$  could be carried away. Loosening of the wrappings made more  $\text{O}_2$  available and accelerated the

disposal of CO<sub>2</sub>, with a consequent decrease in the rate of fall in body-temperature. Tightening of the wrappings produced changes in the reverse direction. The first effect of struggling (fig. 1, C) was to produce more heat and thereby decrease the rate of fall in body-temperature. This quickly reduced the amount of available O<sub>2</sub> and increased the load of CO<sub>2</sub> for excretion. Compensation for these two changes was impossible because of the restraint on respiration and the second response to struggling was an accelerated fall in body-temperature.

Ariel, Bishop and Warren (1943) state that in rabbits no urine is voided during hypothermia. In rats, we find that rather more urine is voided during hypothermia than at normal body-temperature.

#### SUMMARY

1. Hypothermia was produced in rats and rabbits by wrapping the thorax and abdomen with adhesive tape sufficiently tightly to interfere with respiration. The animals were then exposed in the cold room at 2°-4°C.

2. The rate of fall of body-temperature depended on the tightness of wrapping, and the amount of movement of the animals.

3. Rats, in which the body-temperature had been depressed, could frequently regain normal body-temperatures when the wrappings were removed, even while kept at the cold environment. This ability was most marked in young adult rats.

4. When returned to room temperature, newborn rats could recover from body-temperatures as low as 5°C. Rats 30, 60, 80, and 600 days of age, on being unwrapped and returned to room temperature, recovered from body-temperatures as low as 8°, 11°, 15°, and 15°C., respectively.

5. Rats, when wrapped and exposed at high environmental temperatures showed an increase in body-temperature. The neutral temperature for young adult rats was about 31°C.

6. O<sub>2</sub> and CO<sub>2</sub> contents of venous blood were determined in 28 experiments on 12 rabbits during hypothermia. In every case, there was a pronounced fall in O<sub>2</sub> content that was closely parallel to the fall in body-temperature. In every experiment except one (with an abnormally high initial CO<sub>2</sub> content) there was a rise in the CO<sub>2</sub> content of the blood.

7. Two animals were kept at room temperature for 1½ hours after binding. During this time the body-temperature remained normal but the O<sub>2</sub> content of the blood fell and the CO<sub>2</sub> content rose. On being placed in the cold room, these animals exhibited exceptionally rapid falls in body-temperature.

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# SODIUM, POTASSIUM AND PHOSPHATES IN THE CELLS AND SERUM OF BLOOD IN DIABETIC ACIDOSIS

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The *in vitro* studies described in the preceding papers deal with closed systems in which a full accounting can be made. Within this system the total quantities of fixed components, such as chloride, phosphate, potassium and sodium remain the same; only their states and distributions change. Carbon dioxide and glucose can be adjusted. Lactic acid and other products of metabolism can be measured with assurance that they originated within the system. In the circulating blood of the living animal no such exact accounting and control of variables are feasible. The concentrations of potassium, sodium and other stable components in the serum are controlled by extraneous factors. The total quantity of such materials in a given quantity of blood depends not only upon the exchanges between the cells and serum, but also upon the supply and excretion of these materials, the exchanges between tissue cells and extracellular fluid, the volume or dilution of the circulating blood serum, and the addition or removal of metabolic products. There is, however, reason to believe that the reactions of blood *in vitro* are similar in origin and nature to its reactions in the circulating blood.

Guest (1) discovered that at the height of diabetic acidosis the cells of the circulating blood were extremely depleted of organic phosphate, which was restored only gradually over the course of several days during the recovery period. With Rapoport (2) he confirmed the observation of Haldane, Wigglesworth and Woodrow (3) that the organic phosphate of blood diminishes in acidosis induced by administration of ammonium chloride. In pyloric obstruction, on the other hand, they found that organic phosphates rose sharply (2), as they did in extreme renal insufficiency (2). They propose that the red blood cells serve as reservoirs of phosphate that can be made readily available to combat acidosis or when it is needed for purposes of phosphorylation in metabolic processes, especially the combustion of carbohydrate.

The present study was undertaken to verify the work of Guest and Rapoport on diabetic acidosis and to examine further into the mechanisms which control the motions of phosphate.

**MATERIAL AND METHODS.** Blood was drawn from diabetic patients immediately after they were admitted in acidosis and at intervals thereafter. The blood was treated anaerobically. Whole defibrinated blood and serum were analyzed for sodium, potassium, total acid soluble and inorganic phosphate. Serum was also analyzed for CO<sub>2</sub> and chloride. Cell volume was measured. In addition hemoglobin was determined either by measurement of oxygen capacity or total

nitrogen of blood and serum. This permitted the calculation of the water content of cells in all cases, and the water content of serum in all but the first 3 cases in which oxygen capacities, but not serum proteins, were measured. All analytical procedures and methods of calculation have been described in earlier papers of this series (4, 5). Bloods from 3 normal persons and from a few diabetic patients without acidosis were treated in the same manner for comparison.

The patients were treated according to the usual principles observed in this clinic. Two patients received blood transfusions to combat circulatory collapse. There is no evidence that this altered the course of the chemical reactions under consideration. Presumably the foreign cells in their new environment adopted the behavior of the native cells, or else they were sufficiently diluted to cause no distinguishable disturbance.

RESULTS. The essential original analytical data are presented in table 1; functions derived from these data in tables 2 and 3. The first 6 diabetic patients in these tables were studied during recovery from acidosis. Analyses for sodium and potassium were not made on MJ until recovery was well advanced.

It will be noted first that at the height of acidosis cell volume is high, diminishing sharply in the early stages of treatment. Subsequently it fluctuates to a variable degree during recovery. These variations are the product of two processes; changes in volume of individual cells in response to osmotic influences, and alterations of the volume of circulating blood plasma owing to variations of its water content and the state of the circulation. It follows that the cellular content of a given volume of blood on two occasions is not identical, as it is in the *in vitro* experiments. The cell volumes from two observations are not comparable. To overcome this difficulty to some degree in the columns marked "Absolute cell volume" are given the volumes of cells that contain the same amount of hemoglobin as the original blood, calculated by the formula  $\frac{Hb_i}{Hb_f} V_f = \text{"Absolute cell volume"}$  in which  $Hb_i$  and  $Hb_f$  represent the concentrations of hemoglobin in the initial blood and subsequent bloods respectively.

It is assumed in this treatment that the essential properties of the red blood cells do not change in the course of recovery, a somewhat dubious assumption in view of the fact that transfusions were sometimes given and that some degree of hypochromic anemia is a not unusual sequel of diabetic acidosis. Hemoglobin is, however, the most constant element in the red blood cells. In the columns headed "Amounts in cells" are given the quantities of materials in the "Absolute cell volume."

The initial excessive volumes of the red blood cells in acidosis can be attributed to the low pH of the blood and to hemoconcentration. In some instances reductions in the concentrations of sodium + potassium in the serum might seem to contribute; but the concentrations of these elements are far more radically diminished in the cells and this deficit of cellular base is accompanied by extreme depletion of organic phosphate. In every instance  $D_{Na+K}$  of the original blood is less than 1.0. In the process of cellular depletion both potassium and sodium share; there is no evidence that sodium is utilized to spare potassium. In abso-

TABLE 1

*The sodium, potassium and phosphorus of cells and serum in the blood of normal and diabetic subjects*

SUBJECT	DATE	BLOOD			SODIUM		POTASSIUM		ACID SOLUBLE P				SERUM CO <sub>2</sub> VOL- UME PER CENT	
		Sugar mgm. per cent	Hemo- globin per cent	Cell volume per cent	Blood mM. per l.	Serum mM. per l.	Blood mM. per l.	Serum mM. per l.	Inorganic		Organic			
									Blood mM. per l.	Serum mM. per l.	Blood mM. per l.	Serum mM. per l.		
Normals														
SJ				40.3					1.01	1.19	5.89	0.06		
JM				42.5					1.00	1.17	5.69	0.06		
DK				40.0					1.02	1.36	6.25	-0.05		
Diabetics														
1942														
MJ	Nov. 25	1296	12.9	47.0					2.41	3.29	3.78	0		
	Nov. 26	510	19.4	60.0					0.16	0.16	5.02	0		
	Nov. 30		13.0	41.7					0.71	0.65	3.94	0.06		
	Dec. 4		12.0	37.1	88.3	131.5	33.8	3.6	0.75	0.93	5.60	0.03		
	Dec. 9		10.1	35.6	99.0	134.6	41.0	4.6	0.86	1.17	5.99	0		
	Dec. 14		11.3	40.6	94.2	129.3	43.2	5.2	1.19	1.49	7.06	0.03		
NA	Dec. 21	1176	16.1	53.1	67.0	139.5	46.2	4.7	2.06	2.41	4.40	0.07		
	Dec. 23		13.4	40.2	90.0	143.5	39.6	3.9	0.89	1.07	6.54	0.39		
	Dec. 31		11.5	42.2	81.0	127.0	45.2	4.8	0.96	1.29	6.50	0.15		
GA	Dec. 28		11.8	38.6	77.8	120.1	32.9	3.9	2.38	2.43	2.62	0.11		
1943														
OK	Oct. 2	426	14.8	53.6	68.4	120.7	44.2	5.4	1.29	1.41	3.80	0.28	11.1	
	Oct. 3	342	12.1	39.3	82.7	125.8	40.3	3.5	0.31	0.40	2.72	0.11	30.4	
	Oct. 4	325	10.6	31.7	88.6	130.0	37.1	3.1	0.26	0.31	3.61	0.13	38.5	
	Oct. 5	342	11.6	37.2	93.6	133.0	35.0	2.0	0.64	0.91	4.35	-0.01	45.6	
	Oct. 6		10.6	34.5	95.6	133.8	37.0	4.1	0.66	0.81	4.88	0.11	54.7	
	Oct. 7	265	11.2	34.6	97.6	134.5	38.6	4.0	0.91	1.12	4.81	0.18	49.6	
	Oct. 9*	353	10.3	32.0	91.0	133.3	38.1	4.3	1.02	1.17	5.24	0.08	47.3	
	Oct. 11	456	11.8	39.0	88.0	128.4	43.8	4.6	1.04	1.29	6.78	0.06	48.0	
	Oct. 15	364	11.1	36.6	87.0	129.8	43.0	4.9	1.07	1.32	6.41	0.05	48.0	
	Nov. 3	79	12.6	40.2	86.6	133.0	50.3	3.8	1.12	1.61	8.31	0.12	60.3	
	HI	Nov. 1	662	15.4	50.8	68.0	129.1	45.2	3.5	1.06	1.65	3.53	0.85	18.3
		Nov. 2	174	11.9	34.9	94.0	134.3	36.6	3.9	0.50	0.64	3.87	0.11	38.6
	KI	Nov. 5	400	11.9	36.0	90.7	136.0	40.5	4.1	0.91	1.11	5.34	0.05	58.5
Nov. 10		261	13.3	39.1	89.2	140.5	42.3	3.4	0.68	0.90	5.68	0.07	57.4	
Dec. 29		638	16.4	52.3	71.0	148.9	44.2	4.9	1.64	1.95	3.04	0	6.6	
	Dec. 30	136	11.8	33.1	83.5	119.5	28.3	3.2	0.17	0.19	2.49	0.10	24.5	
1944														
KI	Jan. 3	164	11.7	35.4	93.5	138.1	38.2	3.4	0.74	1.15	5.36	0	69.2	
1943														
GU	Nov. 12	89	14.3	44.0	82.5	147.1	47.6	2.0	1.02	1.27	5.72	0.01	73.3	
	Nov. 19	154	14.9	49.2	81.0	134.5	47.0	3.6	0.94	1.18	6.11	0.05	69.5	
CA	Nov. 15	325	15.3	47.4	76.5	129.5	51.4	5.3	0.82	1.10	6.51	0.04	68.6	
AR	Nov. 16	301	11.6	37.5	88.1	134.2	43.8	4.0	1.05	1.19	6.17	0.07	60.4	
	Nov. 23	277	11.8	41.6	86.6	130.0	45.2	5.2	1.00	1.23	6.38	0.03	65.7	
GR	Nov. 18	190	10.5	32.2	100.5	139.2	32.4	4.0	0.97	1.08	4.62	0.04	63.1	
HA	Nov. 22	230	16.4	51.4	74.0	136.0	49.0	3.9	0.86	1.12	5.82	0.01	71.2	

\* The cell volume and cell sodium are so out of line in this blood sample that there is reason to suspect errors in the measurement of cell volume, and probably of hemoglobin.

TABLE 2

*Distribution of sodium, potassium and phosphates between cells and serum, calculated from data of table 1*

SUBJECT	DATE	WATER		NaW		KW		Na + KW		PW			
		Serum per cent	Cells per cent	Serum mM. per l.	Cells mM. per l.	Serum mM. per l.	Cells mM. per l.	Serum mM. per l.	Cells mM. per l.	Inorganic		Organic	
										Serum mM. per l.	Cells mM. per l.	Serum mM. per l.	Cells mM. per l.
Normals													
SJ		93.7	72.7							1.27	1.03	0.2	14.8
JM		93.5	72.1							1.25	1.08	0.2	13.3
DK		93.3	70.8							1.45	0.73	-0.2	15.7
Diabetics													
1942													
MJ	Nov. 25	(93.3)*	76.0							3.52	1.86	0	8.0
	Nov. 26	92.5	72.0							0.17	0.22	0	8.4
	Nov. 30	(93.3)	72.4							0.70	1.08	0.2	9.4
	Dec. 4	(93.3)	71.8	141.0	21.0	3.9	118.5	144.9	133.6	1.00	0.62	0.1	15.1
	Dec. 9	(93.3)	74.6	144.3	46.3	4.9	143.2	149.2	189.5	1.25	0.42	0	16.8
NA	Dec. 14	(93.3)	75.4	138.6	47.1	5.6	141.5	144.2	188.6	1.59	0.92	0.1	17.3
	Dec. 21	(93.3)	73.2	149.5	3.8	5.0	112.8	154.5	151.0	2.59	2.39	0.2	8.2
	Dec. 23	(93.3)	71.1	153.8	14.6	4.2	130.5	158.0	145.1	1.15	0.87	1.3	15.7
	Dec. 31	93.7	76.7	135.5	23.1	5.1	130.5	140.6	153.6	1.38	0.65	0.5	15.2
GA	Dec. 28	(93.3)	73.0	128.7	14.2	4.1	108.1	132.8	122.3	2.61	3.16	0.4	6.6
1943													
OK	Oct. 2	92.2	76.5	130.9	30.3	5.8	101.8	136.7	132.1	1.53	1.55	0.9	6.9
	Oct. 3	93.7	73.7	134.2	21.8	3.8	131.8	138.0	153.6	0.42	6.24	0	6.9
	Oct. 4	94.0	71.3	138.3	0	3.3	154.6	141.6	154.6	0.33	0.18	0.4	11.2
	Oct. 5	93.8	73.3	141.8	36.6	2.1	123.5	143.9	160.1	0.97	0.27	0	11.7
	Oct. 6	94.0	73.7	147.8	31.5	4.3	135.0	152.1	166.5	0.86	0.54	0.4	13.9
	Oct. 7	93.8	72.2	143.4	38.5	4.3	143.3	147.7	181.8	1.19	0.71	0.6	13.6
	Oct. 9	94.2	72.5	141.6	1.7†	4.6	151.7	146.2	153.4	1.24	0.96	0.2	16.2
	Oct. 11	93.4	74.1	137.5	33.5	5.0	142.3	142.5	175.8	1.38	1.09	0.2	17.3
	Oct. 15	93.5	73.9	138.9	30.3	5.3	147.4	144.2	177.7	1.41	0.87	0.2	17.4
	Nov. 3	93.0	73.1	142.9	23.1	4.1	163.5	147.0	186.6	1.73	0.54	0.4	20.5
	Nov. 1	92.9	74.1	139.0	12.0	3.8	115.6	142.8	127.6	1.78	0.67	2.9	6.1
HI	Nov. 2	94.6	70.7	142.0	26.3	4.1	138.0	146.1	164.3	0.68	0.33	0.4	10.8
	Nov. 5	94.8	71.6	143.5	14.4	4.3	146.8	147.8	161.2	1.17	0.78	0.2	14.8
	Nov. 10	93.7	70.7	149.9	13.4	3.6	145.7	153.5	159.1	0.96	0.46	0.2	14.4
KI	Dec. 29	92.6	73.1	160.7	0	5.3	109.6	166.0	109.6	2.11	1.85	0	5.8
	Dec. 30	94.7	69.2	126.1	15.3	3.4	113.0	129.5	128.3	2.04	1.68	0.3	7.3
1944													
KI	Jan. 3	94.1	71.6	146.9	15.8	3.7	142.3	150.6	158.1	1.23	0	0	15.1
1943													
GU	Nov. 12	94.1	72.1	156.4	0	2.2	146.3	158.6	146.3	1.35	0.97	0	13.0
	Nov. 19	93.8	74.0	143.4	34.9	3.8	124.0	147.2	158.9	1.26	0.87	0.2	12.4
CA	Nov. 15	93.9	72.3	137.4	24.5	5.6	142.0	143.0	166.5	1.17	0.72	0.1	13.7
AR	Nov. 16	93.1	73.5	144.4	14.8	4.3	149.6	148.4	164.4	1.28	1.12	0.2	16.4
	Nov. 23	93.2	75.9	139.5	33.9	5.5	133.5	145.0	167.4	1.32	0.90	0.1	15.3
GR	Nov. 18	93.9	71.9	148.3	25.9	4.3	128.1	152.6	154.0	1.15	1.00	0.1	14.3
HA	Nov. 22	93.3	72.7	145.7	21.5	4.2	126.2	149.9	147.7	1.20	0.86	0	11.3

\* Parentheses indicate that serum water was not measured, but the normal average, 93.3 per cent, was used for calculations.

† See footnote to table 1.

TABLE 3

*Distribution coefficients and amounts of Na, K and P in cells, estimated from data in previous tables*

SUBJECT	DATE	D <sub>Na+K</sub>	D <sub>PO<sub>4</sub></sub>	ABSOLUTE CELL VOLUME	AMOUNTS IN CELLS				
					Na	K	Na+K	P	
								Inorganic	Organic
				per cent	mM.	mM.	mM.	mM.	mM.
Normals									
SJ			1.24						
JM			1.16						
DK			2.00						
Diabetics	1942								
MJ	Nov. 25		1.90	47.0				2.06	11.7
	Nov. 26		0.78	39.8				0.20	10.3
	Nov. 30		0.65	41.1				0.99	11.8
	Dec. 4	0.92	1.63	39.4	5.9	33.5	39.4	0.53	18.4
	Dec. 9	1.27	3.02	42.3	19.6	45.2	64.8	0.40	22.1
	Dec. 14	1.31	1.74	46.0	16.3	49.1	65.4	0.98	26.7
NA	Dec. 21	0.98	1.08	53.1	1.5	43.9	45.4	2.89	13.5
	Dec. 23	0.92	1.32	48.2	5.0	44.7	49.7	0.93	23.5
	Dec. 31	1.09	2.13	58.8	10.4	58.9	69.3	0.91	27.7
GA	Dec. 28	0.92	0.85	38.6	4.0	48.5	52.5	2.70	7.9
	1943								
OK	Oct. 2	0.97	0.99	53.6	12.4	41.7	54.1	1.97	11.3
	Oct. 3	1.11	1.79	48.1	7.7	46.7	54.4	0.26	10.3
	Oct. 4	1.09	1.80	44.2	0	48.7	48.7	0.21	15.8
	Oct. 5	1.11	3.56	47.5	12.7	43.0	55.7	0.29	17.2
	Oct. 6	1.09	1.61	48.0	11.0	47.8	58.8	0.59	20.7
	Oct. 7	1.23	1.68	45.6	12.7	47.5	60.2	0.73	19.2
	Oct. 9*	1.05	1.29	46.0	0.6	50.6	51.2	0.99	23.1
	Oct. 11	1.23	1.27	48.7	12.1	51.3	63.4	1.22	25.2
	Oct. 15	1.23	1.61	48.5	10.9	53.8	64.7	0.97	26.0
	Nov. 3	1.27	3.21	47.1	8.0	56.3	64.3	0.57	29.9
HI	Nov. 1	0.89	2.66	50.8	4.5	43.6	48.1	0.78	13.2
	Nov. 2	1.12	2.08	45.2	8.4	44.1	52.5	0.33	15.2
	Nov. 5	1.09	1.50	46.7	4.8	49.1	53.9	0.81	21.3
	Nov. 10	1.04	2.07	45.3	4.3	46.7	51.0	0.46	20.3
KI	Dec. 29	0.66	1.14	52.3	0	41.9	41.9	2.19	9.4
	Dec. 30	0.99	1.22	46.0	4.9	36.0	40.9	0.16	10.5
	1944								
	Jan. 3	1.05	∞	46.4	5.2	47.3	52.5	0	21.8
	1943								
GU	Nov. 12	0.92	1.39						
	Nov. 19	1.09	1.44						
CA	Nov. 15	1.16	1.63						
AR	Nov. 16	1.11	1.14						
	Nov. 23	1.15	1.46						
GR	Nov. 18	1.01	1.15						
HA	Nov. 22	0.99	1.40						

\* See footnote to table 1.

lute quantities the potassium deficit usually exceeds the sodium deficit; but relatively sodium suffers more than potassium. In some instances, in fact, sodium is completely wiped out of the cells. Furthermore, sodium of the cells appears to be reconstituted less rapidly than the potassium. It may even diminish during the early stages of recovery from acidosis, when serum sodium has risen to normal and cellular potassium has been wholly or partly restored.

At the height of acidosis the concentration of potassium in serum is normal or even slightly above normal, despite its low concentration in the cells. With recovery, as the potassium in the cells rises, serum potassium falls sharply, sometimes (for example in OK on Oct. 5) to concentrations as low as those which in other states provoke symptoms of potassium deficiency. This decline of potassium appears also in Guest's (1, 2) studies. At a later stage of convalescence cellular  $\text{Na} + \text{K}$  rises distinctly above the normal concentration, yielding distribution coefficients as great as 1.20 to 1.30, without any consistent effect on cell volume. These high distribution coefficients are not associated with any abnormality of the  $\text{CO}_2$  content of the blood nor any consistent abnormality of water distribution.

The redistribution of cellular potassium and sodium may be accomplished only slowly over the course of several days. The cells may remain partially depleted of base long after  $\text{CO}_2$  has returned to normal and after clinical symptoms and signs of ketosis have disappeared.

The changes of phosphate follow closely the course described by Guest (1). Initially the organic phosphate of the cells is extremely low. In unpublished studies Klinghoffer (6) found that practically all the esters but adenosine-triphosphate had been destroyed. At this juncture inorganic phosphate of serum is usually high and that of the cells may be.  $\text{D}_{\text{PO}}$  is usually greater than 1.0 and may be far greater. This contrasts sharply with the effects of acidification of the blood *in vitro*, which regularly caused the distribution coefficient to fall. When treatment had been instituted and before acidosis had been overcome inorganic phosphate of both serum and cells fell rapidly, causing precipitate changes of  $\text{D}_{\text{PO}}$ , that are consistent neither in direction nor magnitude. Analysis of the amounts of organic and inorganic phosphate in the cells shows that there are two or three processes at work that appear to be independent of one another at this early stage of recovery. Only a small part, usually none, of the inorganic phosphate which leaves the serum at first enters the cells. The fall of serum phosphate must be effected partly by dilution as the fluid stores of the body are built up. Part may be excreted by the kidneys as their function is restored. The remainder must be taken up by cells of other tissues. Loss of organic and inorganic phosphate from the blood cells may continue during this period (cf. MJ, Nov. 25 and 26, and OK, Oct. 2 and 3).

Shortly, however, the synthetic process in the cells begins. This may advance so rapidly that the cellular inorganic phosphate becomes almost extinguished. In those cases in which its concentration falls lowest, serum phosphate is also greatly depleted, giving the impression that the cells in their avidity are removing phosphate from the serum more rapidly than it can be supplied. This

can hardly be the case, however, since the inorganic phosphate of serum is in equilibrium with that of the large pool of extracellular fluid. The amounts withdrawn by the cells, though not inconsiderable, must be small in relation to the quantities in this large mass of fluid. The supply of phosphate during these intervals must be small in proportion to the demand put upon it by all competitors, of which the red blood cells are but one. The slow restoration of organic phosphate and the prolonged low concentration of inorganic phosphate in OK do suggest that extreme reduction of the concentrations of inorganic phosphate in serum may not be altogether without effect upon the ability of the cell to acquire phosphate from the serum. It is during this period in which the inorganic phosphates of serum and cells are fluctuating more or less independently that the distribution of phosphate is most capricious.

DISCUSSION. These studies are not susceptible to such precise analysis and interpretation as the *in vitro* experiments reported in the preceding papers (4, 5). Nevertheless, they contribute unique information. Under the catastrophic impact of diabetic acidosis certain important constituents are explosively discharged from the cells; in the recovery period these are reassembled. Outside of the body such a profound evacuation of vital materials has not been successfully reversed. In the circulating blood stream products of metabolism can be removed and new materials supplied automatically, permitting the blood cells to survive disturbances that are incompatible with life in the rigorously restricted environment of the test tube.

In certain respects the electrolyte disturbances in diabetic acidosis closely resemble those produced *in vitro*. Transfers of neither bases nor phosphates can be correlated with the relative concentrations of these constituents in cells and serum. Paradoxical concentration gradients of all kinds are encountered. The directions in which potassium, sodium and inorganic phosphate move do appear to be linked consistently with the metabolic activities of the cells, and not with these concentration gradients.

By analogy with the *in vitro* experiments and the effects of ammonium chloride (2, 3, 5), it seems logical to ascribe the degradation of organic phosphate and its discharge from the red blood cells in diabetic acidosis to the fall of blood pH. In severe renal insufficiency, however, cellular organic phosphate is usually elevated even in the face of acidosis. Guest (1) has suggested that in renal insufficiency the accumulation of inorganic phosphate in the serum inhibits the effect of acidification. In a system of equilibrium reactions, organic phosphate  $\rightleftharpoons$  intracellular inorganic phosphate  $\rightleftharpoons$  serum inorganic phosphate, general chemical principles would require that accumulation of inorganic phosphate in serum would tend to drive the equilibria to the left. Nevertheless, Halpern (7) was unable to retard phosphorolysis and the loss of phosphorus from cells appreciably by raising the concentration of inorganic phosphate in serum to levels higher than those encountered in renal disease. Furthermore serum inorganic phosphate in severe diabetic acidosis may reach concentrations quite as high as those seen in nephritis. This is exemplified in the first observation on MJ. The high distribution coefficient on this occasion does not indicate that the

higher serum inorganic phosphate seriously obstructed the discharge of phosphate from the cells.

The suggestion that the disturbance of carbohydrate metabolism is involved is equally hard to defend. The glycolytic production of lactic acid by red blood cells does not appear to require the intervention of insulin; outside of the body, at least, it proceeds as rapidly in diabetic as it does in normal blood. In fact diabetic blood was used for many of the experiments described in the preceding paper (5) in order that the glucose in the blood might not become exhausted in the course of the experiments. In general glycolysis and phosphorolysis are inversely related; glycolysis promotes or sustains the synthesis of phosphate esters *in vitro*. There is, nevertheless, some evidence that the metabolism of glucose in the blood cells is not altogether independent of the carbohydrate metabolism of the body at large. Halpern (7) found that the inorganic phosphate of both the cells and serum of a diabetic patient fell after the administration of glucose and insulin. Administration of glucose alone elicited a similar reaction in a normal subject. In neither case did the blood sugar fall to hypoglycemic concentrations. In a group of diabetic patients Dann (8) noted fluctuations of both organic and inorganic phosphate of the cells after administration of glucose and insulin. Even in the absence of acidosis the cellular organic phosphate of diabetic patients varies more than that of normal subjects under comparable conditions. This is illustrated to some extent by the last 7 observations in tables 1 and 2. It is, therefore, possible that in the living organism, through influences that are excluded from the test tube, the phosphorylating system of the red blood cells may be linked with the carbohydrate metabolism of the organism at large.

Losses of base by the cells are still harder to explain. In the experiments on glycolysis *in vitro* as much as 50 per cent of the organic phosphate was broken down and half of the inorganic phosphate thus produced was delivered into the serum. This carried with it, however, no detectable quantities of base. Varying the reaction of the blood by altering  $\text{CO}_2$  tension or addition of sodium bicarbonate likewise leaves the disposition of base undisturbed. In the animal as a whole the excretion of potassium increases in conditions accompanied by destruction of protein and in some states of dehydration. This last reaction was noted by Elkinton, Taffel and Winkler (9, 10) in states of water deprivation, associated with the retention of chloride and sodium. When the organism is under compulsion to excrete solutes in high concentration and at the same time to conserve water—for example when the supply of water is abolished (9, 10) or when large amounts of sodium sulfate are administered with small amounts of water (11)—the reabsorption of sodium and chloride by the renal tubules is increased and the concentrations of these substances in the serum rise. This has a dual beneficial action. By the removal of salt from the urine the kidneys are enabled to excrete in minimal amounts of fluid other solutes with which sodium and chloride compete for water. At the same time the osmotic effect of the retained sodium and chloride forces the cells to give up water to the extracellular fluid, which is thereby protected from extinction. In addition it was

discovered that the cells yielded some potassium over and above the quantities that could be accounted for by losses of protein, when the latter occurred. In these particular experiments the escape of potassium was associated with the retention of sodium and chloride. This association may have been quite adventitious. Potassium depletion may occur also in states of dehydration accompanied by sodium deficiency.

Guest (12) has proposed that the organic phosphates of the red blood cells constitute a reserve phosphate which is released, when it is required, in behalf of carbohydrate metabolism or for the preservation of acid-base equilibrium. This hypothesis, though teleologically expressed, appears to be consistent with the facts. In the light of the present experiments and his own it might be enlarged to include potassium with phosphate. The manner of Guest's statement seems to imply, perhaps quite unintentionally, that the behavior of the red cell differs radically from that of other cells in the body. By assigning to the blood cells a ministerial rôle and naming the tissue cells beneficiaries he almost suggests that their reactions are reciprocally linked. Actually the evidence indicates that their responses in conditions that have been most thoroughly investigated are similar in kind and direction, if not in intensity. From a biological point of view their positions of benefactor and beneficiary may be only adventitious. During acidosis the excretion of phosphate and potassium in the urine is accelerated. Nevertheless, these materials accumulate in the serum. It is highly improbable that the extra potassium and phosphate in the urine and serum together are derived entirely from the red blood cells. During the recovery period serum potassium and phosphorus fall below the quantities in the red blood cells rise appreciably. It may be that potassium and phosphate are delivered more rapidly and reabsorbed more slowly by the red blood cells than by the tissue cells, because of the more rapid metabolism of the latter. The impoverishment of the red blood cells, especially during early stages of recovery, when serum potassium and phosphate are depleted, may reflect a similar deficiency in the tissue cells. The latter can not, however, go to such extreme lengths. Such an interpretation is not at variance with Guest's proposal. It would leave the red blood cells in the position of servant; but their relegation to this position would depend on differences in the intensity rather than the nature of their reactions in comparison with those of the tissue cells. This hypothesis, though consistent with the phenomena of diabetic acidosis does not exclude the possibility that in other conditions the red blood cells may respond distinctively to special agencies. The glycolytic carbohydrate metabolism of these cells is sharply differentiated in some respects from the glycogenolytic metabolism of the tissue cells. Its independence of insulin has already been mentioned. Its obligatory termination in lactic acid also distinguishes it, as does the reputed absence of creatine phosphate from the metabolic cycle. It would be strange indeed if this chemical differentiation was not mirrored in functional differentiation.

From a clinical as well as a purely chemical point of view these phenomena deserve further investigation. Obviously the red blood cell can not be regarded as a mere vehicle for respiratory gases. Its peculiar composition was not con-

ferred upon it merely for the preservation of hemoglobin. Guest (1) has suggested that depletion of serum potassium during recovery from diabetic acidosis may have therapeutic implications. It is necessary to ascertain whether, when and to what extent the red blood cells may be involved as donors or recipients in the changes of serum phosphate that accompany sudden disturbances of carbohydrate metabolism or acid-base equilibrium. The availability and viability of this cell make its reactions peculiarly susceptible to precise investigation. Although in certain particulars it may differ from other cells, as these in turn must differ from one another, these studies indicate that in its general behavior the red blood cell resembles other cells. It is, therefore, unjustifiable to dismiss its reactions as irrelevant to those of the muscle cell, especially when studies of the latter are conducted under conditions in which its integrity is admittedly compromised (13).

*In one respect the red blood cell is especially distinguished from the muscle cell. The former contains variable, often considerable quantities of sodium. Nevertheless it appears to exercise fine discrimination between potassium and sodium. The red blood cell does not use sodium to replace potassium when there is a deficiency of the latter. In some circumstances, indeed, it rejected sodium even when there was a superabundance of this element. The evidence is good that many tissue cells, including muscle cells, may contain small amounts of sodium, which increase under certain conditions of stress. The generalization that sodium is used by the cells to replace potassium when this is depleted rests on far less substantial evidence: the post mortem analysis of tissues, and electrolyte balance studies. The former are open to the objection that potassium and phosphate emerge from the cells explosively in the agonal stage indicating that deprivation of oxygen or other factors connected with death alter profoundly the metabolism, and possibly the permeability of tissue cells. Care is not always taken to minimize these effects and the steady deterioration that follows death. In the balance experiments the assumption is usually made that no chloride enters cells. Although it is known that certain cells invariably contain chloride, it is tacitly assumed that the chloride content of these cells is immutable. Under most circumstances intracellular chloride appears to be relatively stable. It seems to be well established that superfluous chloride introduced into the body is almost, if not altogether, excluded from tissue cells and that chloride lost in most states of dehydration is derived almost, if not altogether, from the extracellular fluid. It can not be inferred that this is an invariable rule. In diarrhea in infants Darrow (14) has recently demonstrated large losses of potassium and chloride in the stools. He has estimated that the cells in this condition take up sodium because of the discrepancies between chloride and sodium balances. This deduction, however, depends on the assumption that there is no change of intracellular chloride in a disorder which involves cells of the gastrointestinal tract which normally contain relatively large quantities of chloride. If the load of sodium in tissue cells does fluctuate present experiments would suggest that these fluctuations are not caused merely by reciprocal exchanges of sodium for*

potassium, but are connected with specialized metabolic reactions, the nature of which remains to be elucidated.

#### CONCLUSIONS

Whole blood and serum of diabetic patients have been analyzed for sodium, potassium, total acid soluble phosphate, inorganic phosphate and water. At the height of diabetic acidosis, as Guest (1, 2) previously showed, the blood cells are extremely depleted of phosphates and base. These are slowly restored during recovery.

The relation of these disturbances to various features of diabetic acidosis and their bearing on cell permeability and transfers of base and phosphorus across cellular membranes has been discussed.

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# THE DISTRIBUTION AND MOVEMENTS OF INORGANIC PHOSPHATE BETWEEN CELLS AND SERUM OF HUMAN BLOOD

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The membranes of the red blood cells of man in the resting state—i.e., when their metabolism is reduced to a minimum by chilling—appear to be impermeable to cations other than hydrogen and ammonium (1). It has been asserted frequently that they are freely traversed by most anions, protein and organic phosphates being the chief exceptions; but this assertion has not been supported by recent observations. Inorganic sulfate added to blood apparently remains entirely in the plasma (2). Inorganic phosphate seems to pass in and out of the cells, not by simple diffusion, but by some more complex process that is linked with the metabolic activities of the cells (3). When lactic acid is added to or accumulates in blood, even if it originated in the blood cells, the major part of it is displaced from the cells into the plasma (4).

The striking differences between the acid pattern of serum and that of cells argue strongly against the free interchange of anions between these media. In the serum the monovalent anions, chloride and bicarbonate, predominate; in the cells their places are largely taken by protein and organic phosphates. The latter, since they are restrained by the cell membranes, must play a part similar to that of the proteins in determining the distribution of ions and water between cells and serum. The nature, state, activity and base-balancing values of these important constituents, however, have not been definitely established. They are heterogeneous and subject to continual transformation. Rapoport and Guest (5), who investigated the acid soluble organic phosphate of blood in a variety of conditions, have attempted to draw up an acid-base balance in the red blood cells. The present studies were undertaken to gain further information about the processes that govern the synthesis and disintegration of phosphate esters and transfers of inorganic phosphate between cells and serum.

**EXPERIMENTAL.** To ascertain the state of inorganic phosphate in serum, observations 15 to 30 of table 1 in a paper by Miller (6) have been used, together with 4 similar unpublished observations by Brown (7) in which concentrations of inorganic phosphate in serum and transudates were compared. In addition Brown measured the inorganic phosphate in ultrafiltrates of serum obtained by means of the Laviertes (8) capsule under anaerobic conditions. In 42 such experiments the original sera, concentrated substrates, and ultrafiltrates were analyzed for protein, calcium and inorganic phosphorus.

In another study defibrinated blood, equilibrated with known tensions of

TABLE 1

*Exchanges of organic acid soluble and inorganic phosphate between cells and serum during autoglycolysis of blood*

NO.	TREATMENT OF BLOOD	BLOOD		INORGANIC P		ORGANIC ACID SOLUBLE P	
		Glucose mgm. per cent	Cell volume per cent	Blood mM. per l.	Serum mM. per l.	Blood mM. per l.	Serum mM. per l.
In refrigerator 18 hrs.							
1.	Original	140	37.9	1.65	1.50	14.25	0.02
	Control	99	37.9	1.20	1.50	14.70	0.00
	at 10 mm. CO <sub>2</sub>	88	37.8	0.98	1.71	14.92	0.09
	at 80 mm. CO <sub>2</sub>	102	40.3	2.90	1.97	13.00	0.01
2.	Original		33.3	2.88	3.63	16.24	0.12
	Control	68	33.2	2.58	3.24	16.54	0.18
	at 10 mm. CO <sub>2</sub>	65	34.6	2.38	3.39	16.74	0.03
	at 80 mm. CO <sub>2</sub>	70	35.2	3.33	3.75	15.79	-0.03
In incubator 4 hrs.							
3.	Original	488	39.0	4.18	5.64	20.12	-0.09
	Control	423	38.7	4.38	5.30	19.92	0.61
	at 10 mm. CO <sub>2</sub>	371	39.8	4.42	5.58	19.88	0.72
	at 80 mm. CO <sub>2</sub>	435	38.5	7.80	9.22	16.50	0.14
In incubator 8 hrs.							
4.	Original	416	47.2	2.10	2.88	31.90	0.12
	Control	305	52.8	11.00	12.60	23.00	0.30
	at 10 mm. CO <sub>2</sub>	282	50.7	8.65	10.62	25.35	1.29
	at 80 mm. CO <sub>2</sub>	321	54.4	15.96	18.00	18.04	1.50
5.	Original	243	42.4	2.13	2.71	19.27	0.04
	Control	135	45.7	5.55	6.30	15.85	-0.02
	at 10 mm. CO <sub>2</sub>	100	42.4	4.83	5.08	16.57	-0.08
	at 80 mm. CO <sub>2</sub>	150	44.6	10.82	11.40	10.58	0.10
6.	Original	212	41.5	2.71	3.18	21.44	0.15
	Control	122	45.3	7.55	7.76	16.60	-0.21
	at 80 mm. CO <sub>2</sub>	114	45.2	12.25	12.60	11.90	0.03
7.	Original	248	44.7	3.94	5.01	22.01	-0.10
	Control	148	46.0	9.20	9.60	16.75	0.15
	at 80 mm. CO <sub>2</sub>	168	46.8	15.15	15.50	10.80	-0.20
At 40 mm. CO <sub>2</sub> and 38°							
8.	Original		39.9	3.00	3.84	18.90	0
	Na <sub>2</sub> CO <sub>3</sub> added		31.0	2.40	3.39	19.50	0.15
9.	Original		45.1	2.71	4.25	22.34	0.01
	NaCl added		34.9	2.93	3.91	22.12	-0.16

CO<sub>2</sub> in air, was allowed to stand in the refrigerator or the incubator for various intervals, in mercury sampling bulbs that prevented entry or escape of gases. At the end of the period blood and serum were analyzed for total acid soluble and inorganic phosphate. In some instances CO<sub>2</sub>, chloride, sodium and potassium were also measured. Cell volume was always measured, together with some functions (oxygen capacity and serum protein, total nitrogen of blood and serum, or dried weight of blood and serum) from which the water of cells and serum could be estimated. In most of the experiments either blood from diabetic patients was employed or else glucose was added to the blood in order to insure against the complete destruction of blood glucose before the end of the experiment. The analytical data from these experiments appear in table 1.

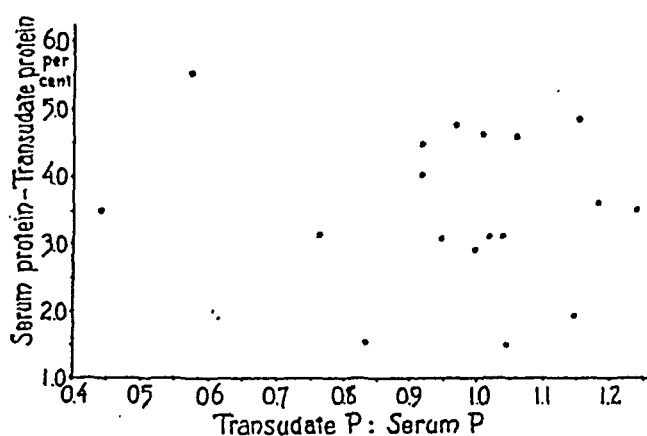


Fig. 1. The distribution of inorganic phosphate between sera and transudates. Phosphate concentrations are estimated in terms of mM. per liter of water in sera and transudates.

Most of the methods used for the treatment and analysis of the blood as well as the methods of calculation have been already described (1, 9). Total acid soluble and inorganic phosphorus were measured by a micromodification of the method of Fiske and Subbarow (10), adapted to the Evelyn photoelectric colorimeter by Hald.

**RESULTS.** It is generally presumed that the inorganic phosphorus of serum is entirely ultrafiltrable. If it is ultrafiltrable and active it should be more concentrated per unit of water in transudates than in serum, which does not appear to be the fact in most published data. In figure 1 the ratio of the concentration of inorganic phosphate per unit of water in transudates to its concentration per unit of water in serum is compared with the difference between the concentrations of protein in the two media. According to the theory this ratio should always exceed 1.0, but actually it is more frequently below 1.0, often far below 1.0. There may be a perceptible inverse relation between the difference in protein and the distribution coefficient, but the differences are too small and the scattering too great to attach any significance to the relation. In figure 2, however, these differences have been greatly exaggerated by comparing ultrafiltrates of

serum with highly concentrated substrates. The protein in the latter varied from 2 (a concentrated transudate) to 24.5 per cent. Again the majority of distribution coefficients lie below 1.0. In fact, of the 42 only one distribution coefficient approaches the magnitude predicted by the Gibbs-Donnan equation. There is a perceptible relation between the distribution coefficient and the concentration of protein in the substrates, roughly defined by the line,  $18.93 - 7.12 D_{PO_4} = \Delta \text{ Protein} \pm \text{s.d.} = 4.32$ . The scattering of the points and the large mean deviation suggest that protein may not be the only force restraining phosphate. Indeed it must be recognized that such a correlation between protein and phosphate may be entirely fortuitous. Protein in such experiments as these serves only as an index of the degree to which the nonfiltrable components of serum have been concentrated. Presumably any other nonfiltrable com-

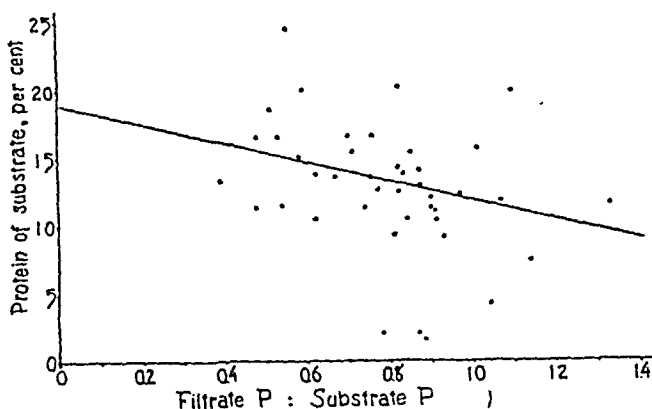


Fig. 2. The distribution of inorganic phosphate between concentrated substrates and ultrafiltrates of serum. Phosphate concentrations are estimated in terms of mM. per liter of water in substrates and ultrafiltrates.

ponent—e.g. calcium—which puts a restraint upon phosphate would be equally concentrated in the substrates. It may be unnecessary to point out that the relation between  $\Delta \text{ Protein}$  and  $D_{PO_4}$  is inverse, whereas, according to the Gibbs-Donnan prediction, it should be direct. There can be no doubt, therefore, that a fraction of the inorganic phosphate of serum is not ultrafiltrable.

In table 2 are data calculated from the analyses recorded in table 1. In all but the first 5 experiments of these tables water of serum and cells was determined. In the first 5 also cell water was estimated, but serum was assumed to contain 93.3 per cent, the amount in average normal serum. In the columns headed "Amounts of P" are given the actual quantities found in cells and serum, obtained by multiplying the concentration of the phosphorus fraction by the volume of cells or serum. From these columns the amounts of organic phosphate broken down and the amounts of inorganic phosphate exchanged between cells and serum can be evaluated.

When blood is allowed to stand its glucose is broken down to lactic acid. This reaction is accompanied by changes in the organic phosphates of the red

TABLE 2  
Data calculated from analyses in table 2

NO.	TREATMENT OF BLOOD	WATER OF		INORGANIC P IN WATER OF		DPO <sub>4</sub>	AMOUNTS OF P			
		Serum per cent	Cells per cent	Serum mM. per l.	Cells mM. per l.		Inorganic		Organic	
							Serum mM.	Cells mM.	Serum mM.	Cells mM.
In refrigerator 18 hrs.										
1.	Original	(93.3)	(72.0)	1.61	2.08	0.77	0.93	0.72	0.02	14.23
	Control			1.61	0.99	1.63	0.93	0.27	0.00	14.70
	at 10 mm. CO <sub>2</sub>			1.83	0	∞	1.06	-0.06	0.06	14.86
	at 80 mm. CO <sub>2</sub>			2.12	5.80	0.36	1.18	1.72	0.01	12.99
2.	Original	(93.3)	(72.0)	3.89	1.92	2.02	2.42	0.46	0.09	16.15
	Control			3.47	1.75	1.98	2.16	0.42	0.12	16.42
	at 10 mm. CO <sub>2</sub>			3.64	0.63	5.78	2.22	0.16	0.02	16.72
	at 80 mm. CO <sub>2</sub>			4.03	3.48	1.16	2.43	0.90	-0.01	15.79
In incubator 4 hrs.										
3.	Original	(93.3)	(72.0)	6.04	2.64	2.29	3.44	0.74	-0.06	20.12
	Control			5.68	4.06	1.40	3.25	1.13	0.39	19.55
	at 10 mm. CO <sub>2</sub>			5.98	3.70	1.62	3.36	1.06	0.43	19.45
	at 80 mm. CO <sub>2</sub>			9.88	7.68	1.29	5.67	2.13	0.09	16.41
In incubator 8 hrs.										
4.	Original	(93.3)	(72.0)	3.09	1.71	1.81	1.52	0.58	0.06	31.84
	Control			13.65	12.75	1.07	5.95	5.05	0.14	22.86
	at 10 mm. CO <sub>2</sub>			11.45	9.08	1.26	5.24	3.41	0.64	24.71
	at 80 mm. CO <sub>2</sub>			19.52	18.80	1.04	8.21	7.75	0.68	17.36
5.	Original	(93.3)	(72.0)	2.91	1.93	1.51	1.56	0.57	0.02	19.25
	Control			6.78	6.30	1.08	3.42	2.13	-0.01	15.85
	at 10 mm. CO <sub>2</sub>			5.44	6.22	0.88	2.93	1.90	-0.05	16.57
	at 80 mm. CO <sub>2</sub>			12.25	13.73	0.89	6.32	4.50	0.06	10.52
6.	Original	93.4	71.2	3.41	2.88	1.18	1.86	0.85	0.09	21.35
	Control	93.0	73.6	8.34	9.92	0.85	4.24	3.31	-0.12	16.60
	at 80 mm. CO <sub>2</sub>	92.9	73.6	13.56	17.13	0.79	6.91	5.34	0.02	11.88
7.	Original	93.1	72.7	5.38	3.60	1.49	2.77	1.17	-0.06	22.01
	Control	93.0	73.5	10.31	11.89	0.87	5.18	4.02	0.08	16.67
	at 80 mm. CO <sub>2</sub>	93.2	73.5	16.64	20.01	0.83	8.24	6.89	-0.11	10.80
At 40 mm. CO <sub>2</sub> and 38°										
8.	Original	92.9	70.4	4.13	2.46	1.68	2.31	0.69	0	18.90
	Na <sub>2</sub> CO <sub>3</sub> added	93.8	62.0	3.62	0.03	121.0	2.34	0.06	0.10	19.30
9.	Original	92.2	70.2	4.61	1.20	3.84	2.33	0.38	0	22.33
	NaCl added	93.5	61.4	4.18	1.77	2.36	2.55	0.38	0	22.12

blood cells. According to Lawaczeck (11), if the blood is kept in the incubator, inorganic phosphate is built up into organic esters during the first 2 or 3 hours, at the end of which time the process is reversed. He found that alkalization favored synthesis, while acidification promoted the breakdown of phosphate esters. Halpern (3) showed that glycolysis favored the synthesis of organic phosphate. These features of intracellular metabolism are illustrated in the tables. In all experiments the breakdown of organic phosphate is greatest in blood exposed to 80 mm.  $\text{CO}_2$  tension; it is invariably smallest at 10 mm.  $\text{CO}_2$ . The "control" bloods, which were kept anaerobically with the 10 and 80 mm. samples, without having been equilibrated, usually fell between the 10 and 80 mm. samples, probably nearer 10, because the bloods were exposed to air in the process of preparation. This confirms Lawaczeck's observation of the effects of acidification and alkalization. At  $37^\circ$  actual synthesis was not demonstrated at the intervals of time chosen. In experiment 3 after 4 hours no phosphorolysis had occurred at 10 mm., though the process was well advanced at 80 mm. In the 8 hour experiments phosphate was broken down in all specimens; the differences between 10 and 80 mm. were only relative. It must be recognized, however, that the differences in reaction in these experiments were also only relative. The tensions of  $\text{CO}_2$  set down in the tables are not those that prevailed at the times of analysis, but those with which the bloods were initially equilibrated. In the interval between equilibration and analysis, while the bloods were in the incubator, lactic acid was formed in all samples. Since there was no provision for its removal, such as there is in the body, by the end of 8 hours even the 10 mm. specimens must have been acid. Evidence of this acidification is found in the swelling of the cells at 10 mm. as well as at 80 mm. In experiments 1 and 2, at refrigerator temperature, which retarded glycolysis, slight actual synthesis of organic phosphate occurred at 10 mm., with destruction of the esters at 80 mm. In experiment 8, in which bicarbonate was added, cellular organic phosphate appears to have increased at the expense of inorganic, even though the blood stood only long enough to permit equilibration.

While phosphorolysis proceeded most rapidly in the 80 mm. samples, the blood sugar fell most rapidly in the 10 mm. samples. This is consistent with Halpern's (3) observation that glycolysis favors the synthesis of organic phosphate. The two processes appear to be inversely related to one another. Alkalization seems to promote glycolysis, while acidification retards it.

In the first 2 experiments in which metabolic reactions were retarded by chilling and in no. 8 in which they were minimized by the short duration of the procedure, in spite of the changes of cellular organic phosphate, no detectable amounts of inorganic phosphate crossed the cell membranes. When, however, the metabolism of the cells was accelerated by raising the temperature to  $37^\circ$ , not only was more phosphate broken down, but the inorganic phosphate thus produced escaped from the cells into the serum. The quantity transferred bears a rough relation to the quantity formed; as the latter increases  $D_{\text{PO}}$ , regularly falls. In this respect it follows the course of the distribution coefficients of  $\text{CO}_2$  and Cl in response to acidification. This is probably no more than a coincidence

since, in experiments 1, 2 and 8,  $D_{PO}$ , moves in the same directions with relation to pH without any transfers of phosphate between cells and serum. The contrast between experiments 8 and 9 is also illuminating. In a preceding paper (9) it was shown that after additions of either bicarbonate or chloride there is a redistribution of both  $CO_2$  and chloride between cells and serum. The distribution coefficients of both rise, albeit to different degrees. Under the same conditions, however, there is no redistribution of inorganic phosphate. Moreover,  $D_{PO}$ , rises to an extraordinary degree after the addition of bicarbonate, but falls after chloride. These divergent changes are occasioned by the osmotic effects of the salt increments and differences in the intracellular metabolic reactions.

DISCUSSION. Halpern (3) showed that phosphate could be made to move from cells to serum against large concentration gradients produced by adding inorganic phosphate to blood. As she pointed out, transfers of phosphate between the two media appear to be linked with the destruction and synthesis of phosphate esters. When cellular organic phosphate is broken down the inorganic phosphate is discharged into the serum; as it is built up inorganic phosphate is withdrawn from the serum. These transfers seem to lag behind the synthesis and destruction of organic phosphate with which they are linked.  $D_{PO}$ , therefore, is determined chiefly by the direction of the metabolic processes in the cells. Although the concentration of phosphate in serum may affect the movements of phosphate, its influence is subordinate to that of these metabolic processes. The distribution coefficient is essentially an indicator of the prevailing direction of these processes.  $D_{PO}$  is greater than 1.0 when the reaction, organic phosphate  $\rightleftharpoons$  inorganic phosphate, is proceeding predominantly to the right; it becomes less than 1.0 when the reaction is moving predominantly to the left. Its magnitude and the amount of phosphate exchanged across the membrane depend upon the speed of the phosphorolytic process.

Although the presence of inactive or nondiffusible inorganic phosphate in serum confuses the quantitative evaluation of distribution coefficients, it can not account altogether for their anomalous behavior. Nondiffusible serum phosphate would make  $D_{PO}$ , consistently too large; but the departures from the laws of simple diffusion equilibrium illustrated in the present experiments and those of Halpern take no consistent direction.

In experiment 5 sodium and potassium of serum, and in experiments 6, 7, 8 and 9, sodium and potassium of both whole blood and serum, were measured. The results of these analyses in experiments 5, 6 and 7 appear in table 3. Experiments 8 and 9 are the same as experiments 6 and 7 of the previous paper (1). In no one of the five were appreciable quantities of sodium or potassium exchanged with the phosphate. In all of these experiments complete glycolysis was avoided by using blood from diabetic patients or blood to which glucose had been added. That potassium may escape from the cells if blood is allowed to stand at incubator temperature was demonstrated by Danowski (12). This reaction is observed, however, only after glycolysis has proceeded to completion and all the blood glucose has been destroyed. In 5 experiments in which blood with normal concentrations of glucose was incubated for from 10 to 16 hours, the

actual amounts of inorganic phosphate in serum increased by 2.3 to 5.3 mM., while potassium increased by 1.9 to 3.5 mM. The escape of potassium does not begin until glycolysis is complete, although the escape of phosphate begins far earlier and the actual amount of potassium transferred is always far less than equivalent to the phosphate.

Conway's (13) theory about the muscle cell depends partly upon the premise that the membrane of this cell allows the free passage of inorganic phosphate, while restraining organic phosphates. A similar presumption is made by Krogh (14). The immobilization of phosphate in the cell is supposed to depend upon the imprisonment of organic phosphate. As this is built up in the cell from inorganic phosphate the latter is replaced by inorganic phosphate which diffuses in from the plasma. Vice versa, inorganic phosphate formed by the breakdown

TABLE 3  
*Sodium and potassium of cells and serum*

NO.	TREATMENT OF BLOOD	Na		K		[Na]*		[K]*		D <sub>Na+K</sub>	AMOUNT IN CELLS	
		Blood mM. per l.	Serum mM. per l.	Blood mM. per l.	Serum mM. per l.	Serum mM. per l.	Cells mM. per l.	Serum mM. per l.	Cells mM. per l.		Na. mM.	K. mM.
5.	Original		139.3		3.3						80.2†	1.90†
	Control		140.0		3.5						76.0†	1.90†
	10 mm. CO <sub>2</sub>		140.0		3.2						80.6†	1.90†
	80 mm. CO <sub>2</sub>		140.5		3.0						77.9†	1.66†
6.	Original	87.9	137.8	47.0	4.5	147.5	24.7	4.8	150.3	1.15	7.3	44.4
	Control	87.9	146.7	47.0	4.3	157.9	23.1	4.6	133.8	0.97	7.7	44.7
	80 mm. CO <sub>2</sub>	87.9	146.1	47.0	4.3	157.3	23.5	4.6	134.1	0.98	7.8	44.6
7.	Original	81.1	139.8	45.6	5.2	150.2	11.7	5.6	131.5	0.92	3.8	42.7
	Control	81.1	142.5	45.6	5.7	153.2	12.1	6.2	125.6	0.86	4.1	42.5
	80 mm. CO <sub>2</sub>	81.1	142.5	45.6	4.0	153.0	15.4	4.3	126.5	0.90	5.3	43.5

\* Concentrations per unit of water.

† Amounts in serum are given in this experiment because whole blood was not analyzed.

of phosphate esters diffuses out of the cells. Conway further proposes that potassium follows phosphate in its movements. So far as the red blood cell is concerned, the major premise on which these hypotheses rest, the permeability of the red blood cell membrane to inorganic phosphate is untenable. Inorganic phosphate added to serum by Halpern (3) did not enter the cells. In fact, phosphate was extruded from the cells in some instances when its concentration in the serum had been increased by such additions. Eisenman, Ott, Smith and Winkler (15) were unable to detect any passage of radioactive phosphate into red blood cells at refrigerator temperature. When cells are active, under circumstances that have been thus far examined, phosphorolysis is generally associated with discharge of phosphate from the cells, while synthesis of phosphate esters is generally accompanied by the entry of inorganic phosphate. The processes are not, however, synchronized as one would expect if these transfers between cells and serum were effected by passive diffusion and distribution

coefficients are produced which are quite incompatible with the concept that the membrane allows the free passage of inorganic phosphate.

It is also evident that movements of potassium are quite independent of movements of phosphate. The immobility of sodium and potassium in these experiments may appear to contradict Danowski's (12) observations. Actually this is not the case. The present experiments differed in some respects from Danowski's. Under somewhat comparable conditions Danowski found from analyses of serum that minute amounts of potassium entered the cells. By methods of calculation similar to his it can be shown that 0.24, 0.27 and 0.85 mM. of potassium left the serum of 80 mm. samples in experiments 5, 6 and 7 respectively. In comparison with the quantities of phosphate transferred these amounts of potassium are negligible. Their only significance lies in the fact that phosphate and potassium move in opposite directions. Sodium figures can not be evaluated with the same precision. For what they are worth it appears that the amounts of sodium in the serum diminished by 0.64, 0.80 and 2.25 mM. If there was any transfer of sodium, therefore, this also moved in the opposite direction from phosphate.

There is substantial evidence that the muscle cell membrane is also impervious to inorganic phosphate (16, 17, 18). In blood large quantities of organic phosphate may be broken down and the inorganic phosphate thus formed may be discharged into the serum unaccompanied by either potassium or sodium. Phosphate and potassium exchanges are not mutually interdependent. In the metabolism of muscle and that of the animal as a whole potassium and phosphate have a tendency to move together. The authors are, however, aware of no large body of data that proves that this association is consistent or that there is a quantitative relationship between potassium and phosphate exchanges. Although in the present experiments these two components appear to be quite independent of one another, it will be shown in a subsequent paper (19) that *in vivo*, under certain influences, their motions appear to be as closely linked as they seem to be in muscle. No clear distinction can be drawn between the two tissues in this respect on the basis of evidence that is now available.

#### CONCLUSIONS

Comparison of blood sera with transudates and with artificial ultrafiltrates reveals the presence in serum of variable proportions of nondiffusible phosphate.

The membrane of the resting red blood cell appears to be impermeable to inorganic phosphate. Movements of this component seem to be linked with the metabolic processes concerned with the synthesis and breakdown of cellular organic phosphate esters.

The cells may lose large quantities of inorganic phosphate without appreciable quantities of sodium or potassium.

Alkalinization of the blood promotes synthesis or delays breakdown of organic phosphate, but accelerates glycolysis; acidification has an opposite effect on both processes.

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# THE MEASUREMENT OF VOLUME OF GAS IN THE DIGESTIVE TRACT<sup>1</sup>

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Gas is acquired by the gastro-intestinal tract by swallowing, by decomposition of intestinal contents, and by diffusion from the blood stream. It is lost by diffusion into the blood stream, by the passage of flatus and by eructation. Of all these processes the only one of importance which is easily measurable is the passage of flatus. Consequently it is difficult to arrive at an accurate estimate of the gaseous exchanges. However some inferences can be made from the daily egestion of flatus and its composition, and from measurements of the gaseous content of the abdomen.

**MEASUREMENT OF VOLUME OF GAS IN DIGESTIVE TRACT. *Theory of method.*** The volume of the gas in the digestive tract may be measured by placing the subject in a closed chamber and having him expire as completely as possible with maximal contraction of the abdominal muscles. The air expired from the lungs does not immediately affect the pressure in the chamber because the heat and moisture it contains are temporarily retained. Compression of gastro-intestinal gas by the expiratory effort, however, decreases the volume of this gas and therefore increases the volume of the surrounding air with a consequent drop in its pressure. This drop in pressure can be made a measure of the change in volume of the gastro-intestinal gas. If its change in pressure is determined simultaneously by a manometer in the stomach, the original volume of the gastro-intestinal gas may be calculated. Instead of using a manometer, an alternative method may be employed in which a given additional volume of gas is introduced into the stomach and the compression is repeated. If the additional gas has volume  $V$ , the original gas has volume  $V_0$ , and the pressure change with expiratory effort is the same after the introduction of  $V$  as before; and the changes of volume of  $V_0$  and  $V_0 + V$  are designated by  $y$  and  $z$  respectively the following relation obtains:

$$\frac{V_0}{V_0 + V} = \frac{y}{z} \quad (1)$$

Since  $V$  is known the measurement of  $y$  and  $z$  give the data necessary for the calculation of  $V_0$  by equation 1.

**APPARATUS.** The apparatus used consisted of a rectangular air-tight tank 105.5 x 130 x 76 cm. made of  $\frac{1}{8}$  inch steel. A door at one end 70 x 111.5 cm. with rubber gaskets permitted easy access. A chair was provided for the subject.

<sup>1</sup> This work was done under contract with the Committee on Medical Research of the office of Scientific Research and Development during 1943-44.

Three inches of concrete were poured on the floor to make it stable. The pressure changes in the tank were measured by means of a metal chamber of 5 liters' capacity to which was connected a segment capsule covered with a membrane of condom rubber. The capsule was made of brass tubing 3.8 cm. in diameter flattened for about 2 cm. on one side. A small concave mirror of 1 m. radius of curvature was fixed to the membrane. A light beam directed through a small window in the tank and reflected back to a scale permitted movements of the membrane to be followed. Calibration of the membrane for volume was determined by injecting into or removing from the tank 50 cc. of air by means of a hypodermic syringe. This deflected the light beam 10 to 15 mm. on the scale with the membrane as usually used.

Because it was protected from air movements in the room the manometer inside the tank was used in preference to a membrane on the tank itself. A small capillary leak was placed in the manometer to avoid steady drifting caused by temperature rise due to heat from the subject. It was convenient also to have a stopcock on the manometer which could be opened to protect the membrane from large pressure changes when the chamber was opened.

**PROCEDURE.** With the subject in the tank it is found that the pressure increases with his inspiration because the inspired air is warmed and wetted. Expiration causes no large immediate change. The subject signals at the end of normal respiration at which time the light beam has stopped and then expires deliberately with maximal contraction of the abdominal muscles, and with care to keep the glottis open so that there is no compression of the pulmonary gases. The observer notes the excursion of the light beam due to the lowering of pressure in the tank consequent to compression of the gastro-intestinal gas. Since each determination requires but few seconds, 5 to 10 are usually made and their average value is used. A given amount of carbonated water is then drunk quickly by the subject and a new series of readings obtained. Or a given amount of air, usually 500 cc., is injected into the stomach, duodenum or rectum, and the readings repeated. The gas volume is then calculated by means of equation 1.

For carbonated water a nationally advertised drink was chosen. The local manufacturer claims to control its  $\text{CO}_2$  content to 3.67 volumes per unit volume within the extreme limits of 3.35 and 3.80 volumes. With 3.67 volumes it should yield about 490 cc. of gas when opened at  $37^\circ \text{C}$ . under atmospheric pressure of  $\text{CO}_2$ . This is taken usually as 500 cc. for convenience in calculating equation 1. When air is injected its volume is used directly.

*Typical example.* A typical determination gave the following deflections in millimeters: 40, 38, 34, 32, 34, 37, 33, 36, before taking  $\text{CO}_2$  in the form of carbonated water and 47, 42, 41, 50, 47, 42, 45, afterward. The initial average is 35 mm. The final is 45 mm. The difference, 10 mm., is due to 500 cc. of  $\text{CO}_2$ . Therefore the initial gastro-intestinal gas volume is  $500 \times 35/10$  or 1750 cc. At this time the manometer sensitivity was 13.5 mm. for 50 cc. change in volume. Therefore the change in abdominal volume for 35 mm. deflection is  $50 \times 35/13.5$  or 130 cc. Assuming a barometric pressure of 760 mm. Hg, the pressure attained by the gastro-intestinal gas was  $760 \times 1750/(1750-130)$  or 820 mm. Hg, an increase of 60 mm. Hg.

*Consistency of deflection.* The example given above shows variation of 10-15 per cent from the mean value. Some subjects frequently do somewhat better, but this is the kind of agreement that can be expected. Most subjects do about as well on the first as on subsequent runs. Closure of the glottis must of course be avoided. It is evidenced by very large and variable deflections.

There was considerable variation in the  $\text{CO}_2$  calibration from day to day particularly in certain subjects. This could be due to differences in the amount of  $\text{CO}_2$  which was released at the time measurements were made, to differences in the muscular effort made by the subjects on different occasions, or to some error in the method. It appeared to be desirable to devise some way of checking those possibilities and also to get some information on the question as to whether or not gas in the stomach is compressed similarly to that contained elsewhere,

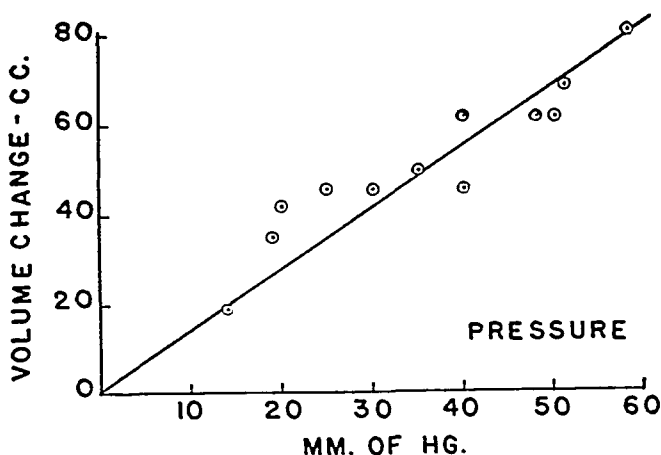


Fig. 1. The decrease in volume of the gastro-intestinal gases resulting from compressional effort by the abdominal muscles plotted against the corresponding increase in pressure in the stomach.

since it must be in order that gas added to the stomach can be used directly for calibration.

*Comparison of different methods.* Although no mutually independent methods were devised, three different combinations of methods comparing gas calibration with the abdominal pressure measurements were used. In the first of these, the subject swallowed a stomach tube terminating in a balloon which was then inflated with 30 cc. of air. The tube was connected to a manometer, and the pressure developed in the stomach with each compression was observed while the change in gastro-intestinal gas volume was determined as usual from the drop in pressure in the closed chamber in which the subject was seated. The subject was instructed to develop a series of increasing pressures. Over small ranges these should be in linear relation, approximately, to the change in volume. This is illustrated by a set of these data plotted in figure 1. Following these measurements the stomach tube was removed, and a series of maximal compressions was then made in the usual way. Frequently this was followed by another series after drinking  $\text{CO}_2$ . The original volume was then calculated, first on

the basis of the pressure-volume data, and second on the basis of the  $\text{CO}_2$  calibration. Comparison of the two quantities thus obtained indicated the consistency of the two methods.

Table 1 shows these relations for a series of measurements on a number of different subjects. The data are divided into good and questionable groups on the basis of the linearity as in figure 1 of the volume to pressure changes by the stomach tube method. An error is possible in this method because a small leak must be left in the volume recorder. If longer times are taken to develop the high pressures, this leak will make the volume change too low for these determinations. In table 1 the so-called good measurements for each subject, if any, are placed separately at the top of each set. The volumes in the column entitled *stomach tube* are calculated as follows: Let the initial gastro-intestinal gas volume be  $V_0$  and its pressure  $P_0$ , the final volume and pressure be  $V$  and  $P$ , then

$$P_0 V_0 = PV$$

$V_0 - V = x$  is measured by the volume recorder. On substitution

$$V_0 = \frac{Px}{P - P_0}$$

The quantity  $x/(P - P_0)$  is the slope of the line of a graph like figure 1. Using this,  $V_0$  is calculated.

The  $\text{CO}_2$  volumes in columns II and IV are obtained from the relation

$$\frac{V_0}{V_0 + 500} = \frac{y}{z}$$

$y$  and  $z$  being the volume changes observed on compression before and after swallowing gas.

The column II gives the initial volumes as calculated not from the original quantities  $z - y$  for each experiment but from the average value of a number of separate  $\text{CO}_2$  calibrations. These are the only values given when no calibration was done during the experiment. Column V gives the ratio of this value to that in column I, while column III gives the ratio of the average  $\text{CO}_2$  volume (column II to the column I volume).

It will be seen that except for the best determinations in the first subject, the average  $\text{CO}_2$  calibration tends to measure somewhat less gas than the stomach tube method. In certain cases, the disagreement between the two methods is rather large, but most of the ratios diverge from the average by less than 20 per cent. The individual  $\text{CO}_2$  determinations, on the other hand, give some very large divergences. This tended to confirm a suspicion that for some reason, the  $\text{CO}_2$  calibration was occasionally very much in error, and that it was better for routine measurements to use the average of several determinations and trust that the subject's performance was sufficiently consistent. However, since this stomach tube method was not sufficiently reliable to provide a good standard, attempts were made to improve it.

Another procedure was carried out in the following way: A series of maximal

TABLE 1

The column entitled *stomach tube* gives the abdominal gas volume as determined from the stomach pressure and the volume change on compression. *Av. CO<sub>2</sub>* gives this same volume from the volume change on maximum compression and the average volume change given to the gas from carbonated water using the same procedure. The next column, *Ratio*, is the average CO<sub>2</sub> volume divided by the stomach tube volume. *Individual CO<sub>2</sub>* volumes are the values obtained from a single trial with CO<sub>2</sub> at the time of the experiment. The final column, *Ratio*, is the ratio of individual CO<sub>2</sub> to stomach tube volume. . . .

SUBJECT	I STOMACH TUBE	II AV. CO <sub>2</sub>	III RATIO	IV INDIVIDUAL CO <sub>2</sub>	V RATIO
	cc.	cc.		cc.	
	970	1040	1.07		
	1360	1480	1.08	1460	1.07
	1120	1060	0.94		
	1160	1320	1.14	1260	1.08
Average.....			1.06		1.06
A	1050	690	0.66	790	0.75
	1170	990	0.85	1500	1.28
	2040	1790	0.88		
	845	830	0.98	735	0.87
	1075	650	0.60		
	2410	1630	0.68		
	1240	1250	1.01	910	0.73
	1230	1450	1.18		
Average.....			0.86		0.91
	620	550	0.89	1700	2.74
	640	430	0.67		
Average.....			0.78		2.74
B	1350	700	0.52	1190	0.83
	650	540	0.83	590	0.91
	860	680	0.79	960	1.12
	630	500	0.79		
	970	640	0.66	450	0.46
Average.....			0.72		0.83
	980	800	0.82	900	0.92
	1230	1120	0.91	2310	1.88
Average.....			0.86		1.40
C	1220	780	0.64	620	0.51
	1740	1480	0.85	1380	0.79
	1880	1820	0.97	1000	0.53
	1350	1380	1.02	3500	2.59
Average.....			0.87		1.10
D	1560	1580	1.01	1150	0.74
	950	1060	1.11	1160	1.22
	1820	770	0.42	600	0.33
	990	900	0.91	1220	1.23
	1490	740	0.50	1100	0.74
	1180	1810	1.54		
	684	630	0.92		
Average.....			0.92		0.85

efforts at compression were made and the volume changes noted. Then a stomach tube with a balloon was swallowed, and the process repeated, both the stomach pressure and the volume change being recorded. Finally the  $\text{CO}_2$  was swallowed while retaining the stomach tube, and a third set of volume and pressure changes were obtained, using again the maximal effort of the abdominal muscles. In these experiments, all the measurements were photographed with a moving paper camera to check the consistency of the pressures. Figure 2 is a typical example of a single determination.

Here the volume record is marked V and the pressure record is unmarked. Beginning on the left, V dipped because the subject inspired. This increased



Fig. 2. Tracing V gives the decrease in volume of the subject when he increases the pressure in the stomach by muscular effort as shown by the unmarked tracing. A permanent slow leak in the volume recorder designed to keep it near equilibrium position causes the drift upward of V while the subject holds his breath preparatory to starting the compressional effort. The drift in this case is undesirably large.

the pressure in the recording chamber because the inspired air was warmed and wetted. The pressure in the stomach was raised slightly at this time by the breathing effort. Following expiration the subject held his breath, and the volume recorder drifted toward the equilibrium it would have had with no breathing. At the second break in the records, the subject compressed his abdominal content by expiratory effort. In consequence the stomach pressure rose, and a corresponding volume decrease occurred. Finally the subject inspired deeply to terminate the maneuver. This process was repeated 7-10 times, and the average values were used for calculation. The drift in the volume recorder is a source of error but this is usually compensated for roughly by a drift in the opposite direction following the displacement due to compression.

The results of these experiments are given in table 2. The column headed *Average  $\text{CO}_2$*  gives the initial volume calculated from average  $\text{CO}_2$  calibrations for this subject and the volume changes obtained without the stomach tube.

TABLE 2

The columns *Av.* and *Ind. CO<sub>2</sub>* give, respectively, the abdominal gas volume as determined by average CO<sub>2</sub> calibrations and individual trials in the particular experiments. Columns *V-initial* and *V-final*, respectively, are the gas contents before and after drinking CO<sub>2</sub> calculated from pressure changes in the stomach and volume changes of the abdominal gas on compressional effort. The column, *Diff.*, is *V-final* minus *V-initial* which should equal 500 cc., the gas yielded by the carbonated water. *P<sub>0</sub>* and *P* are the pressures developed in the stomach by maximal efforts before and after drinking carbonated water. The volumes *y* and *z* are the changes in volume obtained by compression of the abdominal gas before and after taking the stomach tube.

SUBJECT	AV. CO <sub>2</sub>	IND. CO <sub>2</sub>	V-INITIAL	V-FINAL	DIFF.	P <sub>0</sub>	P	y	z
			cc.	cc.	cc.	mm. Hg	mm. Hg		
F		1600	925	1160	235	67	70	34	74
		725	980	1460	480	60	68	33	71
		710	760	1260	500	72	74	41	66
		1480	1410	1740	330	65	71	88	111
		625	815	1260	445	55	65	16	55
		615	1190	1650	460	51	64	62	75
Average.....		960	1010	1420	410	62	69		
G	920	920	750	1290	540	84	75		75
	570	970	480	730	250	81	79	47	46
	700	715	685	1530	845	92	84	57	74
	960	700	630	1070	440	102	103	79	74
	1590	1560	1340	1630	290	104	98	130	140
	1730	1260	1090	1660	570	104	110	142	131
	1270	2460	1060	1270	210	95	96	104	118
Average.....	1110	1220	865	1310	450	95	92		
H	930	760	1430	1970	540	96	118	162	160
	900	1030	1440	1840	400	107	127	159	177
	1010	1650	1310	1670	360	121	125	178	180
	780	580	830	1560	730	132	131	138	123
Average.....	905	1005	1250	1760	510	114	125		
K		1710	500	645	145	104	104	56	60
		1160	675	945	260	98	100	55	77
		2240	700	830	130	92	96	65	76
		960	580	790	210	84	90	57	58
Average.....		1520	614	800	185	96	98		
Average of F, G, H, and K.....	1035	1160	980	1330	400	89	93		

The column headed *Ind. CO<sub>2</sub>* is the initial volume calculated from the change given by the CO<sub>2</sub> in this particular experiment. *V Initial* is the initial volume calculated from the stomach pressure and volume changes before taking CO<sub>2</sub>; and the *V Final* is the volume afterwards. The next column headed *Diff.*,

which gives the difference between these two volumes should equal 500 cc., the  $\text{CO}_2$  yielded by the carbonated water. The columns headed  $P_0$  and  $P$ , respectively, give the pressures developed in the stomach before and after taking  $\text{CO}_2$ . There is ordinarily a resting pressure of about 10 mm. Hg in the stomach which is not included in these values. This residual pressure is not altered markedly by the added gas. The last columns,  $y$  and  $z$ , give the changes in volume on compression before and after taking the stomach tube respectively.

It will be seen that, in the case of the first subject in the table, the average difference in volume is just over 400 cc. instead of 500 cc., a deficiency of 100 cc. Since  $V$  initial is about 1000 cc. and  $V$  final is about 1500, this quantity is about 10 per cent of  $V$  initial. An error of 10 per cent in  $V$  initial and -10 per cent in  $V$  final will account for 250 cc. in error which is about the greatest for this subject. In this case, then, the results are consistent with the conclusion that measured volumes are the true values within about 10 per cent. Since the  $\text{CO}_2$  difference is always too low, i.e., less than 500 cc., there may be an indication here that the gas elsewhere in the abdomen is not being subjected to the same pressure as that in the stomach. On the other hand, there is considerable chance of loss of some of the  $\text{CO}_2$  in the stomach, as well as in swallowing carbonated water around a stomach tube. Consequently, this discrepancy cannot be considered a serious one.

Subjects G and H give results similar to those found in subject F, but K gives lower differences. Subject K is fairly consistent, but the average  $\text{CO}_2$  addition is only about 185 cc. This subject may not treat the stomach gas in the same way as the rest of the abdominal content.

Columns  $P_0$  and  $P$  show that different subjects are capable of developing quite different pressures in the stomach by maximal effort. The average of all subjects is over 80 mm. Hg and the highest individual value is 130 mm. Hg. Despite the pressure, there is seldom any difficulty in retaining the gas in the stomach during compression. Only one subject, F, compresses very differently without the stomach tube. It will be seen in table 2 that his values of  $y$  (cubic centimeters compressed without the tube) are sometimes only about one-half of  $z$  (cubic centimeters compressed with the tube). The other subjects usually give approximately equal compressions.

In comparing the individual  $\text{CO}_2$  determinations in column 2 with the stomach tube volumes, it will be seen that the agreement has to be exact whenever  $P_0 = P$  and the measured  $\text{CO}_2$  difference is 500 cc. This result is closely approximated for the third experiment of the first subject. Otherwise, when  $P_0 = P$  approximately, but the added gas is less than 500 cc., column 2 will be too large. This is because the  $\text{CO}_2$  was assumed to give 500, but the stomach tube gave a change in volume commensurate with a lesser amount.

Comparing column 2 with column 3, it will be seen that occasionally the  $\text{CO}_2$  volume diverges quite considerably from that obtained from the pressure change. However, the average volumes for each subject are approximately equal for the two methods. At the bottom of the table are given the averages for the four best subjects F to H.

It has been concluded, taking the results of tables 1 and 2 together, that the average of at least several CO<sub>2</sub> calibrations is much more reliable than the individual daily values. Consequently this is the method which was used in following the gas content over long experimental periods. This is, of course, equivalent to assuming that a given subject always develops the same pressure in a compressional effort and that, in consequence, his gas volume at any given time is measured by the resulting change in volume. It will be seen in the last column of table 2 that this assumption is not altogether justified, but there is reason to believe that the subject, since he is more comfortable, will perform more consistently without the stomach tube.

The approximate equality of the average gas volumes as determined by the pressure and by CO<sub>2</sub> calibration leads to the conclusion that both methods usually measure all the abdominal gas fairly well. The CO<sub>2</sub> method involves the presence of a disproportionate amount of gas in the stomach. Consequently, if this gas were not compressed approximately equally to that contained elsewhere, the two methods would not give similar results. Exceptions to this may be found in subject K.

Another rough check on the method of volume measurement and the carbonated water calibration is provided by data obtained by making volume measurements before and after the addition of air by tube into the rectum or duodenum. In table 3 the column headed *Site* indicates where the air was injected and the next column gives the amount in cubic centimeters. In the succeeding column under *Calculated* are the added air volumes calculated from compression measurements using the average CO<sub>2</sub> calibration for a five day period about the middle of the interval (several weeks) covered by the experiments. Finally in the last column are calculations of the subjects, initial volumes calculated from compression measurements using the injected air for calibration, rather than the average CO<sub>2</sub>.

Subjectively, at least, the measurements made after injecting gas per rectum are more difficult to make and possibly therefore less accurate because it requires effort to retain the gas while it is being compressed. It will be seen that there are considerable divergences of the measures from the actual air volumes, but the average values are in fairly good agreement except for subjects H and J whose measurements are too high. Although added air will absorb CO<sub>2</sub> from the tissues it is not likely that this factor can account for more than a slight increase over the injected volume in the short interval allowed between injection and measurement. Consequently the high values can be attributed to inaccuracy of the CO<sub>2</sub> calibrations in those subjects. Whether this is due to calibrations being too distant from the measurement or to inability of these subjects to compress gas equally in all parts of the gastro-intestinal tract is not known. It appears, however, on the whole that the calibration made on the stomach is equally valid for the duodenum and colon.

The final column in table 3 makes possible an estimate of the average gas content based on air rather than on CO<sub>2</sub> calibration. This quantity will be considered now.

TABLE 3

Volume measurements following injection of air usually 500 cc. by rectal or duodenal tube as indicated. The column *added air calculated* gives the measured volumes of the added air using an average CO<sub>2</sub> calibration. The final column gives the subject's initial volumes as calculated from the added air rather than carbonated water. Only those cases in which 500 cc. was injected are averaged in the first and second column.

SUBJECT	SITE	ADDED AIR VOLUME CC.		SUBJECT'S INITIAL VOLUME CC.
		Actual	Calculated	
H	Duodenum	500	550	890
	Duodenum	500	880	590
Average.....		500	715	740
L	Duodenum	500	180	2150
	Rectum	500	680	330
	Rectum	500	610	510
	Rectum	500	900	350
	Rectum	500	550	500
	Rectum	500	660	590
Average.....		500	580	740
G	Duodenum	500	520	1690
	Rectum	500	220	3450
	Rectum	500	850	570
	Rectum	500	440	1380
	Rectum	500	840	480
	Rectum	750	900	890
Average.....		500	575	1410
J	Rectum	500	770	1000
	Duodenum	500	940	630
	Rectum	500	740	1130
	Rectum	350	590	920
	Rectum	500	1020	660
Average.....		500	865	850
F	Duodenum	500	415	1010
	Rectum	500	325	880
	Rectum	500	610	710
	Rectum	500	530	870
	Rectum	500	565	750
Average.....		500	490	845
M	Rectum	500	680	690
	Rectum	500	160	2340
	Rectum	500	330	830
	Rectum	500	660	820
Average.....		500	445	1170

Average initial volume of all subjects 960 cc.

*Gastro-intestinal gas content.* Table 4 gives the maximum, minimum, and average gas volumes from a large number of measurements by the CO<sub>2</sub> method on a group of subjects. These are young men in the middle twenties with no apparent digestive disorders. The average content for all is 1330 cc. It will be noted, except for the last subject, that the averages are about midway between the maximum and minimum, and also that the maxima are about twice the minima. This indicates that a given subject varies symmetrically over a range from his minimum to twice his minimum value.

In previous work with different subjects (Blair, Bates, and Fenn, 1943) the average volumes determined in the same way for 3 young women weighing about

TABLE 4

The abdominal gas contents *Max.*, *Min.*, *Av.* are the maximum, minimum, and average values obtained from about 40 individual determinations on each subject over a period of 2 months. The numbers under the heading, *Time of Day*, give relative abdominal gas volume before breakfast, lunch, dinner, and during the evening, respectively. *Post-defecation* volumes are values determined within two hours after defecation relative to the daily average. *No. of Det.* gives the number of determinations on which the defecation results were based.

SUBJECT	WEIGHT	VOLUME CC.			TIME OF DAY				POST-DEFECTION	
		Max.	Min.	Av.	1	2	3	4	Vol.	No. of det.
	<i>lbs.</i>									
K					108	97	99	99	95	25
L	130	1100	550	780	98	91	100	112	88	21
H	140	1840	820	1270	105	94	101	100	83	10
U									92	3
G	160	2380	1180	1680	108	98	100	94	105	26
T						96	100	114	108	6
F	185	1750	920	1320	100	103	95	92	105	7
J	135	1740	960	1420		103	95	100		
M	170	2600	800	1490						
Average.....				1330						

120 pounds was 615 cc. while for 10 men weighing about 160 pounds it was 1175 cc.

Those in table 4 are the same subjects whose volumes determined by air injection are given in table 3. It will be seen that the average by air injection, 960 cc., is appreciably less than the average by carbonated water, 1330 cc. It may be that the carbonated water volumes are somewhat higher on account of CO<sub>2</sub> loss. In any case, air calibration will tend to be low because the injected gas will gain CO<sub>2</sub> while the carbonated water calibration will tend to be high because CO<sub>2</sub> will be lost. On the whole, however, it appears that the method of measurement is fairly consistent as regards variations of procedure. Occasional high values, volumes in excess of two liters, must be regarded with suspicion but there appears to be no reason to question the conclusion that gastro-intestinal

tract ordinarily contains about a liter of gas. Although this is more than has usually been supposed to be present it is a quantity quite in keeping with the view that at least one-half liter of air is swallowed per day, that  $N_2$  diffuses very slowly from the gut and that it requires about a day for it to be lost as flatus and that the swallowed air residue is ordinarily diluted with about equal volume of formed gas.

*Variation of gas volume in relation to time of day and to defecation.* Also in table 4 under the heading of *Time of Day* are given average readings on a number of subjects at different times of day. The numbers 1, 2, 3, and 4 indicate, respectively, before breakfast, between breakfast and lunch, between lunch and dinner, and during the evening. The measurements usually were made at least 3 hours after meals. In each case, the value given is the average of 30-50

TABLE 5

Egested flatus collected by rectal tube and balloon for 6 consecutive days. Diet on days 1-5 inclusive, Army K ration less one half canned goods biscuits and sugar with the exceptions of high carbohydrate lunch on the third day and high protein lunch (1 lb. of beef steak) on the fifth day. Diet on day 6, high carbohydrate.

Subject J.

	DATE					
	1	2	3	4	5	6
Period						
8-12 a.m.	0	0	10	0	0	58
12-6 p.m.	0	0	90	10	14	0
6-10 p.m.	30	2	28	20	18	14
10 p.m.-8 a.m.	105	10	135	15	50	270
Total, cc.....	135	12	263	45	82	342

determinations. The numerical value is the average for the particular time of day as a per cent of the average for the whole day. In two subjects measurements made before and within one hour after meals were approximately the same.

It was expected that defecation would give a decrease in gas volume, but this appears not to be the case. Table 4 gives average compression within two hours following defecation in percentage of the average of the same number of readings at other times of the same days. It will be seen that the post-defecation results are not consistently smaller, indicating that losses during defecation are soon made up.

*The routes of exchange of gastro-intestinal gas, and the constitution of flatus.* The daily egestion of flatus has been shown to be just above 500 cc. as an average from 5 subjects by Beazell and Ivy (1941). Fries (1906) gives a value of 1 liter. Data given in table 6 for subject M give estimated daily values averaging 2700 cc. It may even approach 0 according to subject J, table 5. These divergent data indicate that the volume is quite variable.

TABLE 6

Egested flatus collected by rectal tube and balloon for the waking hours of the day. These data were obtained while the subject was on controlled diets containing about 3000 calories derived from fat 133 grams, carbohydrate 420 grams and protein containing 4.87 grams nitrogen, from either egg or soy bean as indicated. The first two sets of data are on the first and third days of a four day period on egg protein. The following three sets of data are for the first, third and fourth days of a succeeding four day period on soy bean protein. The diet on the 4 consecutive days on soy bean is typical except for protein and consisted of applesauce 100 grams, butter 39 grams, dressing 40 grams, orange juice 400 cc., coffee, 5 cups, and soy flour biscuits. Although this is not markedly reflected in the daily egestion of gas the soy bean diet caused subjectively at least a great deal more flatulence particularly on the first day of a period. On changing to egg diet the subjects became quite comfortable, losing the urge to pass gas to obtain relief from distention.

The gas analyses are for the samples opposite to which they are placed in the tables except when otherwise indicated. The average composition of the samples is CO<sub>2</sub>, 23.4; O<sub>2</sub>, 3.3; H<sub>2</sub>, 52.5; CH<sub>4</sub>, 3.1; N<sub>2</sub>, 23.3; all in per cent.

TIME	GAS	TIME	GAS	TIME	GAS		
Diet 4% egg protein, first day							
8:45-9:45 a.m.	cc. 40	5:40	cc. 65	9:00	cc. 65		
10:15	35	6:40	70	10:00	170		
2:30 p.m.	95	8:10	30	11:00	135		
3:45	105	8:15	65	12:00	40		
4:25	145	8:25	35	Total.....	1270		
5:00	115	8:35	70	Estimated daily egestion 2000 cc.			
Diet 4% egg protein, third day							
			ANALYSIS OF SAMPLE (%) 8-12 p.m.				
			CO <sub>2</sub>	O <sub>2</sub>	H <sub>2</sub>	CH <sub>4</sub>	N <sub>2</sub>
8-11 a.m.	160		17	7	52	2	23
1:15 p.m.	110						
2:15	95						
4:15	180						
5:20	145						
7:00	130						
8:00	75						
9:00	160						
10:00	150						
11:00	120						
12:00	75						
Total.....	1400						
Defecation at 8:00 p.m.			Estimated daily egestion 2100 cc.				
Diet 4% soy protein, first day							
8-10:30 a.m.	80	5:00	cc. 325	10:30	cc. 140		
11:30	230	6:00	120	11:00	105		
12:35 p.m.	260	7:05	300	11:30	120		
1:35	135	8:00	0	12:00	165		
2:40	115	9:15	0	12:30	60		
4:10	360	10:00	90	1:00	10		
			Total.....	2615			
Estimated daily egestion 3700 cc.							

TABLE 6—*Concluded*

TABLE 6—Continued							
TIME	GAS	TIME	ANALYSIS OF SAMPLE (%)				
			CO <sub>2</sub>	O <sub>2</sub>	H <sub>2</sub>	CH <sub>4</sub>	N <sub>2</sub>
Diet soy protein, 3rd day							
	cc.						
8:00-10:15	70		28	2.1	56	1.2	14
12:40 p.m.	110		17		69		15
2:10	140		34		47		31
3:15	220		43	2.6	39	1.6	19
4:20	340						
5:20	275						
7:30	345						
9:30	460						
10:30	160						
11:30	60						
2:00 a.m.	0						
Total.....	2180						
Defecation at 9:30 p.m.			Estimated daily egestion 2900				
Diet soy protein, fourth day							
9:12 a.m.	100						
3:00 p.m.	200		10	3.4	62	0.3	27
4:30	550		29		60		16
6:00	180						
7:45	280		20		63		18
10:00	150		13	2.6	45	2.0	40
12:30	60						
Total.....	1520						
Defecation at 12 noon			Estimated daily egestion 2350				

It is supposed by some that the daily egestion of flatus is about equal to the average content. This view would be justified if it could be shown that the gas is acquired principally by aerophagy and that it required one day on the average to pass through the gut or that there was an exchange equivalent to this. Analyses of flatus which gave nitrogen contents similar to that of air would lend some support to the hypothesis that the gas egested as flatus is acquired principally by swallowing because it is known that nitrogen is taken up relatively slowly by the blood stream. However, the constitution of flatus is most variable and is probably like air in nitrogen content only rarely. Consequently the stressing of any given factor such as aerophagy is likely to be misleading, and it is important to consider the whole complex possible events effecting the transfer of gas to and from the gastro-intestinal tract.

It is inevitable that air will be swallowed in saliva and with food and beverages. The beaten white of one egg, for example, contains about 175 cc. of air. This is an extreme case but there is a tendency particularly with cereals to introduce considerable quantities of gas in preparing them for the table. Con-

sequently it seems probable that considerable air may be swallowed per day and that an estimate of about 1 liter would not be excessive in most cases. Swallowed air can be passed into the bowel according to Cannon (1911) and McIver, Benedict and Cline (1926), and in the present work air injected into the stomach has been found in considerable quantity in the small bowel by x-ray photography. Since it is thought that little gas is formed in the small bowel the conditions effecting the absorption of air from both the stomach and small bowel should be similar. These will be considered now.

Air reaching the stomach will find itself at a pressure about 8 to 10 mm. Hg above atmospheric. Table 8 shows the approximate relation between the gaseous tensions in the blood and stomach for air recently swallowed, and after the  $O_2$  and  $CO_2$  have approached equilibrium. That these gases will tend to equilibrium before there is much exchange of nitrogen is shown by the ratios of the absorbabilities of intestinal gas by the small bowel of the cat as determined by McIver, Redfield, and Benedict (1926),  $CO_2:169$ ,  $O_2:100$ ,  $H_2S:79$ ,  $H_2:7$ ,  $CH_4:4$ ,  $N_2:1$ . Due to the relatively great diffusibility of  $CO_2$  there may be an intervening stage at which the stomach air has become sufficiently diluted with  $CO_2$  to reduce the outward gradient of  $N_2$  below its initial value. It will be seen, however, that an outward gradient of at least some 60 mm. Hg ordinarily will exist. Despite its slow absorption rate  $N_2$  can be expected to leave the upper part of the gastro-intestinal tract by diffusion to at least some extent.

An estimate of this can be made from studies of  $CO_2$  loss from the stomach. By the method of volume measurement used in the present work two-thirds of the increase of volume caused by the ingestion of 500 cc. of  $CO_2$  is lost in about 50 minutes. Since some  $O_2$  will have been gained, the time-constant for the loss of  $CO_2$  (time for loss of about  $\frac{2}{3}$  initial volume, assuming it to be exponential) will certainly be less than 50 minutes. Direct measurements by Ylppö (1917) of the fall in concentration of nearly pure  $CO_2$  injected into the stomach in volumes from 750 to 1050 cc. showed that it reached a concentration of 5.3 per cent or approximate equilibrium with alveolar  $CO_2$  in about 1 hour. The actual rate of loss is not determinable from these data but the concentration had fallen to 14 per cent in 45 minutes in one case and 12.9 per cent in 35 minutes in another, i.e., to within about 9 per cent and 8 per cent respectively of its final value. Dilution with  $O_2$  is again a factor but a time constant of about 20 minutes is probably consistent with these results. Ylppö showed by the same method that  $O_2$  concentration fell to about 24 per cent in one hour, i.e., to within 7 per cent of its equilibrium value of 17 per cent. This is consistent with a time constant of roughly 40 minutes, about twice as great as that of  $CO_2$  as it should be to agree with the ratios of McIver and others given above.

If the time-constant for pure  $CO_2$  loss is taken as 20 minutes that of pure  $N_2$  under the same conditions will be  $20 \times 169$  minutes. Since, however, the gradient of  $N_2$  from the stomach is about  $\frac{1}{12}$  atmosphere the diffusion rate will be decreased 12-fold. Consequently 100 cc. of  $N_2$ , a probable average stomach content, will be absorbed at the rate of  $100/(12 \times 20 \times 169) = 0.0025$  cc. per minute or 3.6 cc. per day. This amount is negligible compared with that swal-

lowed. The essential correctness of the order of magnitude of this estimate is supported however, by the work of Schoen (1925) who could detect no significant loss of injected  $N_2$  or even the more diffusible  $H_2$  from the small bowel of the dog in an hour.

It is also substantiated by the work of Fine, Sears, and Banks (1935) who found that the empty small bowel of the cat when tied at both ends and filled with pure  $N_2$  lost 16 per cent in volume in 24 hours. Of the remainder about 20 per cent may have been  $O_2$  and  $CO_2$  acquired from the blood. Consequently the loss of  $N_2$  was not much in excess of 30 per cent per day, even from the small bowel where conditions for loss are more favorable than in the stomach as regards the wall area in relation to volume.

In the light of these considerations it does not seem probable that the stomach and small intestine together will lose more than a moderate fraction of the  $N_2$  of swallowed air. Nevertheless Fine and Levenson (1933) showed that in the starving cat mechanical obstruction might lead to distention or not. This result shows that sufficient  $N_2$  can be lost by diffusion to compensate for that quantity acquired by swallowing, providing it is not excessive.

However, if the diffusibility of  $N_2$  is of advantage in achieving the loss of gas from the upper part of the gastro-intestinal tract it is of disadvantage in the lower part where other gases are formed by chemical action. Here according to the flatus analyses of table 6, for example, the formed gases  $CH_4$ ,  $CO_2$ , etc., have diluted  $N_2$  to the extent that its percentage is about 25 and its partial pressure about 200 mm. Hg. Consequently there is a  $N_2$  gradient from blood to colon of more than 500 mm. Hg and if the permeabilities of the tissues concerned are similar throughout the tract, there will be inward diffusion of  $N_2$  per unit area of the wall of the large bowel about 8 times as fast as the outward diffusion at the stomach and small bowel.

It will be seen that any region of the gut at which a gas such as  $CO_2$  or  $CH_4$  begins rapidly to form will be practically a vacuum with respect to  $O_2$  and  $N_2$  of the surrounding tissues. If the formed gas is produced in a limited region it will tend to expand into adjacent regions until it is covering sufficient wall that its diffusion outward into the blood is equal to its rate of formation. Meanwhile  $O_2$  and  $N_2$  will diffuse in to dilute the formed gas and to reduce in consequence its outward diffusion. The bubble grows and continues to do so until an equilibrium is reached at which  $O_2$  and  $N_2$  are balanced with the tissues and sufficient viscus wall is covered that the formed gas despite its dilution is able still to leave as fast as it is formed. This equilibrium is probably attained rather seldom under normal conditions. Colon flatus for example is often passed when the  $N_2$  content has reached only about 25 per cent or about one-third of its equilibrium value. With obstruction of the bowel and distention, however, it will be seen according to Hibbard's data (1936) that  $N_2$  ranges around 70 per cent, close to equilibrium whether the small or large or both bowels are involved.

This high value is due largely to swallowed air but since there are substantial amounts of formed gas, some inward diffusion of  $N_2$  probably is also responsible in part. That there is any other mode of exchange of gas such as secretion is

most unlikely as Wangenstein and Rea (1939) showed in dogs that the exclusion of swallowed air by cervical esophagostomy made possible complete occlusion of the terminal ileum for an average survival period of 36 days without distention.

In view of the above considerations it does not seem possible to draw any exact conclusions regarding the history of  $N_2$  appearing in flatus but the low rate of exchange combined with the existence of an outward gradient in the upper part of the gut and an inward gradient in the lower part make it appear possible that

TABLE 7

These data on the same subject as those in table 6 show that he does not pass exceptionally large volumes of flatus when on normal diet. Not as much  $N_2$  is egested per day in these cases. This indicates that more of the  $N_2$  of swallowed air is absorbed by the blood when gas formation is low than when it is high.

Subject M.

TIME	GAS	ANALYSIS OF SAMPLE (%)				
		CO <sub>2</sub>	O <sub>2</sub>	H <sub>2</sub>	CH <sub>4</sub>	N <sub>2</sub>
Normal diet high in carbohydrate						
	cc.					
8:30 a.m.-5:20 p.m.	30					
5:30 p.m.-6:30 p.m.	150					
6:30-7:30	30					
7:30-10:00	120	12.4	5.6	22.2	8.5	52.5
10:00-12:00	70					
Total.....	400	Estimated daily egestion 600 cc.				
Normal diet						
8:00 a.m.-10:00 a.m.	110					
10:00 a.m.-2:00 p.m.	0					
2:00 p.m.-7:00 p.m.	0					
7:00-11:00	30					
11:30-1:00 a.m.	60					
Total.....	200	Defecation 2:00 p.m. Estimated daily egestion 280 cc.				

no great error will be made ordinarily in equating all the  $N_2$  of flatus to swallowed air in normal individuals under average conditions. The data, however, do not support the idea that this is a general rule.

If it is assumed that subject M of table 6 produced gas throughout the whole day at the rate observed while collecting, the daily production varied from 2000 to 3700 cc. with an average over 6 days of 2700 cc. Since the average concentration of  $N_2$  was 23.3 per cent this corresponds to an average passage of 630 cc. per day or to the swallowing of 800 cc. of air. Fries' figure of one liter passed per day with an  $N_2$  content of 59.4 per cent gives a similar value. The values for Beazell and Ivy's subjects are not determinable but they must be smaller

although perhaps not necessarily too small to account for swallowed air. Subject J of table 5, however, egested less than 50 cc. of gas per day on 2 days out of 6. These volumes are much less than that of the swallowed air could possibly be. This subject in fact appears to be capable on occasion of losing practically all of the formed and swallowed gas by way of the lungs. Subject M also (table 7) on normal diet egested certainly no more than 200 cc.  $N_2$  which is about 400 cc. less than his average on the special diets of table 6. Presumably this 400 cc. was lost by diffusion.

This result raises a question therefore as to whether the  $N_2$  exchange is at all regular. Does subject J, for example, always lose swallowed  $N_2$  nearly completely by absorption from the stomach and small bowel? If so the  $N_2$  passed as flatus when egestion is large must come principally from the blood stream through the wall of the large bowel. If not the variable egestion of  $N_2$  must come through

TABLE 8

The approximate partial pressures in millimeter Hg of swallowed air before and after it has equilibrated with the blood compared with those of the venous blood when the barometric pressure is 760 mm. Hg. With  $O_2$  and  $CO_2$  at equilibrium there is a gradient outward from the stomach for  $N_2$  of nearly 70 mm. Hg. A similar relation is to be expected in the small bowel and even in the large bowel when no gas is being formed.

	AIR IN STOMACH		GASEOUS TENSION OF VENOUS BLOOD
	Newly swallowed	At equilibrium	
$O_2$	144	40	40
$CO_2$	1	46	46
$N_2$	578	637	570
$H_2O$	47	47	47
Total.....	770	770	703

variable motility of the small bowel,  $N_2$  being passed into the large bowel when motility is high, before it has time to be absorbed. It seems probable that both motility and diffusion may be important and the latter in particular is influenced by gas formation which will be considered now.

Subject M of table 6 egested as flatus an average of 1400 cc. of  $H_2$  per day. Herbivorous animals have been shown to produce large quantities of  $CH_4$  and  $H_2$  and to lose it principally by way of the blood and lungs. Boycott and Damant, (1907) for example, showed that the goat produces in this way about 10 liters of these gases per day while Washburn and Brody (Alvarez, 1940) collected 300 liters of  $CH_4$  per day from the dairy cow. Parsons (1930), however, found only traces of combustible gases in the expired air of man and Schoen's experiments mentioned above showed very slow loss of  $H_2$  from the small intestine of the dog.

Since Parsons' results may have been due to the choice of subjects in whom the production of combustible gases was low at the time, and since the conditions for diffusion are not likely to be markedly different in herbivores and man

and since their blood concentrations are low it seems reasonable at present to suppose that  $H_2$  and  $CH_4$  will be lost in substantial quantities through the lungs whenever their production is high. In subject M, for example, with  $H_2$  of the flatus averaging 52 per cent, its gradient to the blood is in excess of 350 mm. Hg. Accordingly, in consideration of its diffusibility it should be lost to the blood about  $\frac{1}{50}$  as fast as pure  $CO_2$ . Consequently, it appears probable that this subject's production of  $H_2$  was at least several liters per day.

In the case of  $CO_2$  the daily passage of 630 cc. cannot be more than a small fraction of the total production since its average concentration 23.4 per cent gives a pressure gradient of about 130 mm. Hg from colon to blood. It is scarcely possible that the production can be less than many liters per day and easily conceivable that it may amount to 25 liters or more. In any case it is clear that for this subject at least, the daily turnover involves much more formed than swallowed gas. Nearly all the analyses in table 6 give  $CO_2$  contents in excess of that required for equilibrium with the blood. In these cases also it is probable that  $CO_2$  production was high, and that most of it was lost through the lungs. From these considerations it seems probable that the turnover of intestinal gas in man is not infrequently at least 10 liters per day and may often be considerably higher.

The large production of gas in the bowel makes possible an explanation of the marked differences in passage of flatus by different subjects and at different times by the same subject. Air swallowing is probably not widely variable in a given subject under normal conditions but certain foods and various states of nervous tension often lead to large changes in the volume of flatus passed. It seems likely that the difference is due principally to changes in motility of the gut. An active large bowel in which gas is being formed will tend to pass it frequently while an inactive bowel will tend to retain it sufficiently long that the easily diffusible elements will be absorbed in large part by the blood. Consequently the daily volume of egested flatus bears no necessary proportionality to the volume of formed gas. When the tendency for expulsion of gas is low the daily volume will approach the lower limit determined by the amount of residual swallowed air and its  $N_2$  content will ordinarily be high. When the tendency for expulsion is high the proportion of diffusible gas will be high and the daily volume will be limited only by production but it probably never approaches closely this value. Contrary to the usual interpretation of the evidence, the average gas content of the bowel may often be high when the elimination is low and low when elimination is frequent and copious.

#### SUMMARY AND CONCLUSIONS

The gas volume of the digestive tract is measured in fair approximation by determining its change in volume during compression by maximal expiratory effort along with its consequent change in pressure, or by determining the changes in volume with maximal expiratory effort before and after addition of a known volume of gas. The average content of male subjects is about 1 litre. There are no consistent variations with time of day. Defecation gives no significant

reduction. Collection of flatus by rectal tube for 6 consecutive days in one subject with army K ration as the principal diet shows daily egestions from as low as 12 cc. up to 340. Another subject collecting all day except during sleep gave values ranging from 200 cc. on normal diet up to 2600 cc. on diets with soy bean for protein. The  $N_2$  contents were about 100 cc. and 600 cc. respectively. The exchanges between the gastrointestinal tract and the blood of swallowed air and formed gases are discussed. The conclusion is reached that the volume of egested flatus is largely dependent on the motility of the gut. When this is high, large amounts of gas are passed with the  $N_2$  highly diluted with formed gases but of a relatively large total content which possibly approximates the amount swallowed. When motility is low small volumes of gas of high  $N_2$  concentration but of low total  $N_2$  content are egested. These results indicate that even though swallowed  $N_2$  is absorbed slowly it may be absorbed almost completely.

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# THE PRESENCE IN BLOOD OF A PRINCIPLE WHICH ELICITS A SUSTAINED PRESSOR RESPONSE IN NEPHRECTOMIZED ANIMALS

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Both essential and experimental hypertension are believed by many to be caused by a pressor agent which emanates from the kidneys and circulates in the blood stream. The presence of this hypothetical substance in the blood of hypertensive patients or animals has never been unequivocally demonstrated. The most direct evidence for the existence of a humoral pressor agent is the experimental work of Solandt, Nassim, and Cowan (1). These investigators cross-transfused equal amounts of blood between hypertensive dogs and normotensive, bilaterally nephrectomized dogs and observed that the blood pressures of the latter were acutely and moderately elevated, presumably by a pressor substance in the blood from the hypertensive animals.

The present paper is concerned with the outgrowth of certain observations made in a series of experiments which were performed in an attempt to demonstrate the presence in blood of a humoral substance which could be capable of causing hypertension.

For a preliminary study, a simple and direct approach was decided upon. Because of the very great pressor sensitivity of the pithed cat, it was felt that this preparation might be used to detect a possibly greater pressor activity in the plasma from a hypertensive animal or patient than in that from a normotensive one.

**METHODS.** For test animals, cats weighing 1.8 to 4 kgm. were used. Each cat was given 1.3 mgm. of atropine sulfate, and was anesthetized with pentobarbital sodium (30 mgm./kgm., intraperitoneally); artificial respiration was instituted after cannulation of the trachea. Both vagi were severed and one carotid artery ligated. The other carotid artery was cannulated and mean blood pressure (BP) recorded with a mercury manometer. The animal was pithed by forcing a bluntly pointed  $\frac{1}{8}$  inch diameter rod through one of the optic foramina and the foramen magnum and passing it down the spinal canal. The BP usually leveled off after 15-20 minutes at 40-70 mm. Hg. Injections were made through a 6 inch length of plastic tubing<sup>1</sup> (1 mm. OD) inserted into one of the femoral veins.

**RESULTS.** The intravenous injection into the prepared cats of 2-5 ml. of the plasmas obtained from normal and hypertensive dogs and patients caused acute moderate elevations in BP which subsided in 3-10 minutes. There was no consistent difference between the pressor responses of normal and hypertensive plasmas.

<sup>1</sup> "V-1 tubing." A. C. Balfour Associates, Englewood, N. J.

On the assumption that the "hypertensive" pressor substance might be in the same protein fraction in which renin would be expected to be found, 15 normal and hypertensive dog and human plasmas were precipitated at 0.6 saturation with ammonium sulfate, the precipitate taken up in water, dialyzed, centrifuged, and the supernatant fluid concentrated *in vacuo*. Again the various extracted plasmas could not be differentiated on the basis of their pressor responses.

In the next series of experiments the bilaterally nephrectomized pithed cat was used as the test animal. This preparation was chosen in accordance with the concept that the kidneys could function as an "antagonist" to the hypertensive pressor agent. Supporting evidence for this belief is the observation that blood from hypertensive dogs will cause an elevation of BP only in dogs which have been bilaterally nephrectomized (1). In dogs made hypertensive by unilateral clamping of the renal artery, removal of that kidney is followed by a prompt disappearance of the hypertension, presumably due to the presence of the opposite normal kidney (2). Bilateral nephrectomy in dogs made hypertensive by renal artery clamping or by wrapping the kidneys in silk is *not* followed, in most instances, by subsidence of the hypertension. (In table 1 are presented data showing the BP changes following bilateral nephrectomy in 9 hypertensive dogs.) From these findings it would seem to follow that: 1, a remarkably stable "hypertensive" substance was produced in the kidney and, following bilateral nephrectomy continued to circulate in the blood stream and exert its pressor effect until the death of the animal from uremia; 2, the kidney is the only organ which functions to any great extent as an antagonist to the hypertensive pressor agent. Consequently, as a working hypothesis, it was tentatively held that, if a sufficient quantity of the hypertensive pressor substance were to be injected into the blood stream of a nephrectomized animal the BP should become elevated and remain so for hours or days. In addition, a smaller amount of the same substance might be expected to exert only a very prolonged (3), if not sustained, pressor effect in a sensitive nephrectomized acute animal preparation. Regardless of the validity of such reasoning it was decided to use cats which had been bilaterally nephrectomized 2 days before with the hope that they would have had sufficient time for the "elimination" of the hypothetical inhibitor and still not be so far into uremia as to constitute a poor test preparation. Following nephrectomy under pentobarbital sodium anesthesia the cats were given 30-50 ml. of saline subcutaneously; the same amount was given the following day. The animals were allowed no food or water following nephrectomy. On the day of the test the cats were anesthetized and pithed as described.

Before presenting the results obtained with the nephrectomized preparations it is pertinent to relate certain events which form the background for an entirely unpredicted observation. A cat was observed to be very sick and a few minutes later it died. Because of a temporary difficulty in obtaining cats, and rather than allow the animal to be a total loss, blood was withdrawn from its heart and venae cavae, heparin was added and the plasma obtained by centrifugation. With full cognizance of the fact that such a plasma could not be regarded as a normal control sample of cat plasma, it was nevertheless included among several dog and human plasmas which were being processed the same day. Each

plasma, at that time, was treated in the following way: 2 volumes of saline were added, the mixture adjusted to pH 4.0 with glacial acetic acid and the precipitate

TABLE 1\*

DAYS FOLLOWING NEPHRECTOMY.....		0	0.8	1.2	1.8	2.2	2.8	3.2	3.8	4.2	4.8	5.2	5.8
Dog 2764. Kidneys wrapped in silk. Hypertensive 3 mo. Ave. B.P. 200 mm. Hg	MBP BUN	210 24	135 132		175 213	Died. Dark blood in abdomen							
Dog 2866. Kidneys wrapped in silk. Hypertensive 3 mo. Ave. B.P. 190 mm. Hg	MBP BUN	200 19			140 145	80 206	Died. Serosanguinous fluid in abdomen						
Dog 2876. Kidneys wrapped in silk. Hypertensive 3 mo. Ave. B.P. 190 mm. Hg	MBP BUN	205 20	200 169		195 279	Died 1.5 hr. later							
Dog P102. Kidneys wrapped in silk. Hypertensive 12 days. Ave. B.P. 190 mm. Hg	MBP BUN	205 42	175 85	210	180 173	Died. Purulent peritonitis							
Dog P108. Kidneys wrapped in silk. Hypertensive 13 days. Ave. B.P. 180 mm. Hg	MBP BUN	170 19	170 56	170	170 105	170 183	Sacrificed for another experiment						
Dog P127. Renal arteries constricted. Hypertensive 5 mo. Ave. B.P. 200 mm. Hg	MBP BUN	180 32	185 69	185	185 120	175 183	195 183	200	195 261	195	145	Died. Pneumonia	
Dog P137. Renal arteries constricted. Hypertensive 5 mo. Ave. B.P. 190 mm. Hg	MBP BUN	180 15	205 49	185	180 95	195 137	200	215	205 188	200	215 260	210	Died
Dog P201. Rt. neph. Left kidney wrapped in silk. Hypertensive 1 mo. Ave. B.P. 240 mm. Hg	MBP BUN	240 15	260 65	265	245 138	Died							
Dog P211. Rt. neph. Left kidney wrapped in silk. Hypertensive 1 mo. Ave. B.P. 200 mm. Hg	MBP BUN	185 41	195 79	180	195 130	185 177	205	175	180 232	200	150	110 318	Died

\* Changes in femoral arterial mean blood pressure (MBP) in mm. Hg and blood urea nitrogen (BUN) in mgm. per cent of trained hypertensive dogs following bilateral nephrectomy (sodium pentobarbital anesthesia). Dogs 2764, 2866 and 2876 were allowed food and water *ad libitum*. All others were allowed nothing by mouth, but were given subcutaneously 150-200 ml. physiological saline postoperatively and daily thereafter. Vomiting and dehydration were greatly reduced in the latter, treated group.

removed by centrifugation. The supernatant was dialyzed against cold running tap water for 2 days, centrifuged and the supernatant concentrated *in vacuo* to one-half the original plasma volume.

In figure 1 (injection no. 3) is shown the pressor response to the injection of 2 ml. of the treated plasma into a pithed cat which had been bilaterally nephrectomized 48 hours before. A most remarkably sustained pressor response resulted. From a base line of 37 mm. Hg the BP of the test animal rose to 90 mm. one hour after injection and was 100 mm.  $2\frac{1}{2}$  hours after injection. No gross change in heart rate was observed. At this time another 2 ml. of the treated cat plasma was given (injection no. 4, fig. 1); the pressure further increased to 147 mm. and leveled off at 142 mm. one-half hour after the second injection. About one hour later the pressure began to decline slowly and five hours after the first injection it was 104 mm. Hg.

Here, then, found in a dead cat's plasma was a substance which exhibited those physiological properties *hypothetically* required of the humoral hypertensive agent. When 2 ml. of the same plasma was injected intravenously into a non-nephrectomized pithed cat the pressor response was relatively small and was not sustained. Plasmas obtained from 7 different, normal, living cats either processed in the same manner or unmodified—failed to cause a sustained pressure elevation when injected into “48-hour nephrectomized” pithed cats. Similarly, plasma obtained from one cat killed by acute bleeding and from another killed by a blow on the head failed to give a sustained pressor response (fig. 2).

Since the property of being able to cause a sustained pressure elevation (hereinafter referred to, for the sake of brevity, as “activity”) was found in the plasma of the sick cat which had died and not in plasmas obtained from normal, living cats or from those which were killed acutely, it was, of course, desirable to repeat the original experiment, if possible, and obtain plasma from cats which had died as the result of illness. An opportune accident revealed a method by which one could make a cat ill and also predict reasonably well the time it would die so that its blood could be drawn immediately thereafter. A normal cat had been inadvertently exposed to an unknown amount of a “DDT” solution<sup>2</sup> and a few days later it became listless and died. Heparinized plasma from blood obtained from its heart and venae cavae, when tested on a nephrectomized pithed cat preparation, proved to have well sustained pressor activity, similar to that shown in figure 3. By the trial and error method it was found that approximately 3.2 ml. of the same DDT solution per kgm. body weight, given intraperitoneally, usually caused the cats to die within 15–24 hours. Plasmas from cats which had been killed with DDT exhibited no detectable difference in activity from plasmas subsequently obtained from cats which became ill and died of “natural causes.” In most instances ascitic fluid (30–50 ml.) taken from the abdominal cavity of cats given DDT solution intraperitoneally was found to possess a moderate amount of activity after being centrifuged and dialyzed.

*Factors affecting the production of the active pressor principle.* As mentioned earlier, plasma obtained from the blood of normal, healthy cats killed acutely by bleeding or by a blow on the head did not possess any sustained pressor activity.

<sup>2</sup> “Toxite” spray to which 8 per cent “DDT” (1,1,1-trichloro-2,2-bis[p-chlorophenyl]-ethane) had been added.

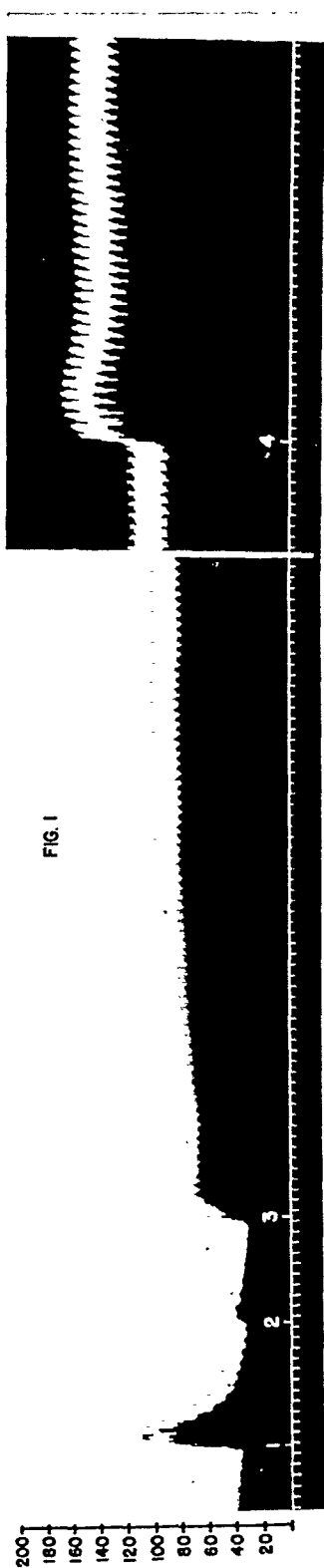


FIG. 1

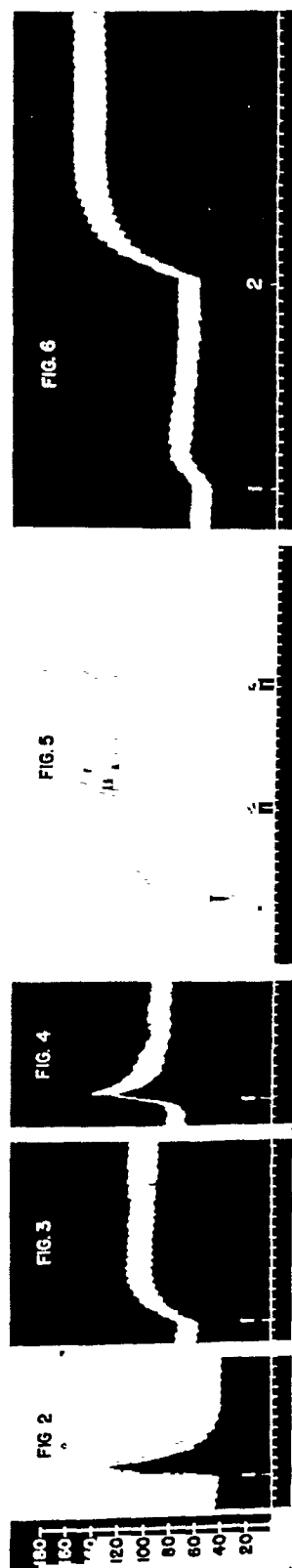


FIG. 5

FIG. 4

FIG. 3

FIG. 2

FIG. 6

Fig. 1-6. Mean blood pressure tracings of pithed cats nephrectomized 2 days before. Ordinate scales—mm. Hg. Time marker—1 minute.

Fig. 1. I.V. injections: 1, angiotonin; 2, 2 ml. concentrated plasma from hypertensive patient; 3, 2 ml. concentrated plasma from dead cat (see text); one hour segment of record removed at break; 4, 2 ml. of same cat plasma given at 3 (32ZC-36).

Fig. 2. I.V. injection of 4 ml. of concentrated dialyzed blood plasma from cat killed by blow on head (32ZC-40).

Fig. 3. I.V. injection of 2.5 ml. dialyzed plasma from terminal blood of cat killed with DDT (32ZC-101).

Fig. 4. I.V. injection of 4 ml. of 7 per cent solution of acacia (32ZC-51).

Fig. 5. Repeated intravenous injections (1, 2, and 3) of 2 ml. of dialyzed plasma from terminal blood of cat killed with DDT (32ZC-126).

Fig. 6. I.V. injection: 1, cat kidney extract containing renin; 2, 2 ml. of active terminal cat blood plasma which contained same amount of renin as injection 1 (32ZC-177).

On the other hand, the ability to cause a sustained pressor response in "2-day nephrectomized" pithed cats has been demonstrated in each of 56 different plasmas obtained from cats, either killed with DDT or having died of natural causes. One cat, depancreatized and allowed to die in coma, also gave an active plasma. In general, then, the cats which died more or less "gradually" gave active plasmas while those which died acutely gave inactive plasmas.

It would be expected that if the kidneys were the source of the pressor substance the plasmas from the terminal blood of bilaterally nephrectomized cats would be inactive. Five cats were given DDT solution 8-12 hours after nephrectomy and the post-mortem blood was drawn as usual. None of the plasmas exhibited any sustained pressor activity. The plasmas from 5 bilaterally nephrectomized cats which died spontaneously 24-26 hours postoperatively were also inactive. It was concluded therefore that the presence of the kidneys was necessary for the production of the pressor substance found in the terminal blood.

Because of the fact that a period of hypotension must have preceded the cats' deaths (one DDT cat's mean BP was found to be 20 mm. Hg 5 minutes before death), it was decided to bring about the death of the cats in a more controlled manner by bleeding them repeatedly until the BP reached a low level, and allow the hypotension (35-50 mm. Hg) to continue for varying periods of time or until the animals died. In other experiments a different method was used for maintaining the low BP. The cats were given an anticoagulant (Pontamine fast pink, 150 mgm./kgm., intravenously); the femoral artery was cannulated and connected, by means of tubing, to a flask suspended 47 cm. (35 mm. Hg) above the level of the cannula. This kept the animal's BP at a constant low level until death.

Both anesthetized and unanesthetized cats were subjected to the above procedures. With the first method the blood samples withdrawn in the process of lowering the BP and the terminal blood sample were tested on "2-day nephrectomized" cats for sustained pressor activity. As one example (fig. 7, nos. 1, 2, 3), the plasma from the control blood (mean blood pressure 140 mm. Hg) exhibited a very slight degree of activity.<sup>3</sup> Plasma obtained from the next two bleeding samples (average BP 60 mm. for 25 min.) had somewhat greater activity. The terminal blood, in this instance obtained after 2 hours of hypotension (50 mm. Hg), had the most activity. Essentially the same results were obtained in each of four similar experiments. There appears to be a minimum period of time for maintaining the animals in a hypotensive state before activity can be demonstrated in the blood plasma (see table 2).

The same experimental procedure was repeated using cats which had been nephrectomized immediately before bleeding to hypotensive levels, and in none

<sup>3</sup> It is frequently found that blood drawn from a cat which has been anesthetized with pentobarbital sodium a short time before possesses a slight degree of sustained pressor activity. Samples drawn 30-60 minutes later have been found to be inactive. No activity has been observed in the plasma of blood drawn from unanesthetized normal cats or anesthetized cats which have previously been nephrectomized.

of the terminal blood samples obtained from 4 nephrectomized cats was sustained pressor activity found (fig. 7, no. 6). The minimum requirements for the production of the pressor agent therefore appeared to be: 1, a period of hypotension, sustained for 30 minutes or more, and 2, the presence of kidneys in the animal during the period of hypotension. The data are summarized in table 2.

*Factors affecting the response of the test cat preparation to the injection of the sustained pressor principle.* Normal healthy cats which had been anesthetized, pithed, and otherwise prepared in the same manner as the "2-day nephrectomized" preparation usually failed to exhibit any sustained pressure rise when injected with plasma which was known to be active. In the occasional instance in which a small sustained pressure rise was observed, it could not be regarded as being

TABLE 2

*Sustained pressor activity of terminal blood plasma from nephrectomized and non-nephrectomized cats which had been subjected to various degrees of hypotension*

EXPERIMENT	ANESTHETIZED	KIDNEYS	LEVEL OF HYPOTENSION	DURATION OF HYPOTENSION	SUSTAINED PRESSOR ACTIVITY OF TERMINAL BLOOD PLASMA
			<i>mm. Hg</i>	<i>minutes</i>	
Z-242	No	Intact	40	20	±
Z-199	No	Intact	40	25	+
Z-261	No	Intact	50	30	+
Z-178	No	Intact	60-40	45	++++
Z-281	No	Intact	40	75	+++++
Z-212	Yes	Intact	40	20	—
Z-179	Yes	Intact	50	90	++
Z-229	Yes	Intact	45	90	+++
Z-286	Yes	Intact	50	120	+++
Z-213	Yes	Removed	30-20	30	—
Z-211	Yes	Removed	45	105	—
Z-187	Yes	Removed	40	135	—
Z-287	Yes	Removed	40	300	—

necessarily due to the active pressor material present, since normal cat plasma or even 7 per cent acacia solution have several times been found to give similar responses (fig. 4).

The dramatic difference in the responses of the normal pithed cat preparation (kidneys intact) and the "2-day nephrectomized" preparation is illustrated by the example shown in figures 8 and 9. Two milliliters of fresh heparinized plasma from the terminal blood of a cat killed by the DDT method, when injected into a nephrectomized preparation caused the 65 mm. rise in BP seen in figure 8. Two milliliters of the same plasma injected into a normal (non-nephrectomized) preparation caused the response seen in figure 9. Another normal pithed cat was given 3 successive injections, 5 ml. each, of a different active plasma, and in all instances the BP promptly returned to essentially its base level after an acute pressure rise. The same active plasma given in only

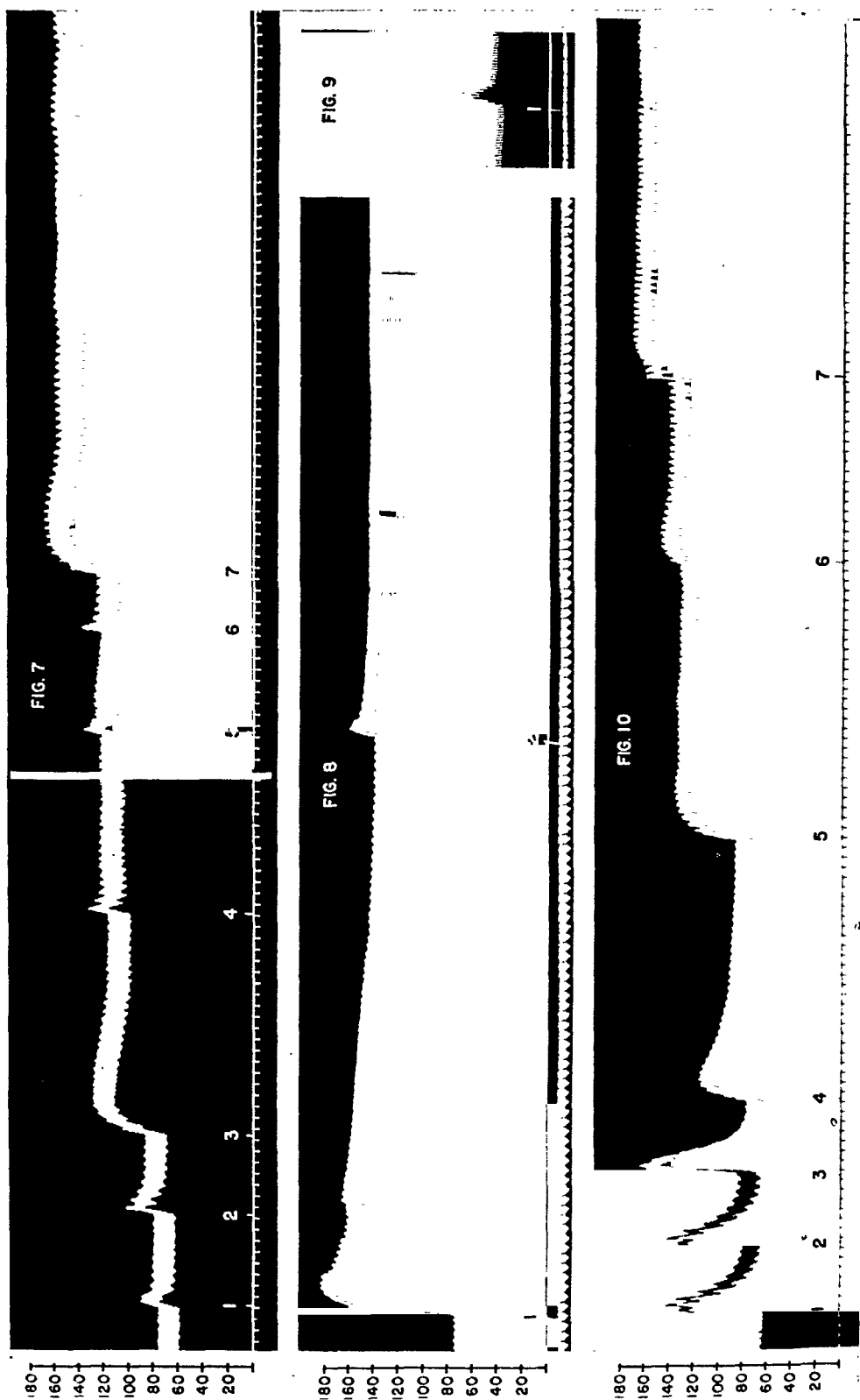
2 ml. amounts to a nephrectomized preparation gave a stepwise and progressive elevation of the BP as shown in figure 5.

The pressor responses in the "2-day nephrectomized" cats were usually well sustained at or near the level to which the BP originally rose; occasionally the BP continued to climb slowly for 30 minutes or more as in figure 1. With repeated injections the pithed cat's BP approached what appeared to be the maximum sustained level for this preparation (105-180, ave. 150 mm. Hg), and additional injections of active plasma frequently caused a further increase which was sustained at a level somewhat lower than the initial acute rise (fig. 5, injection no. 3).

In 7 out of 71 "2-day nephrectomized" preparations used so far in this study, the injection of plasma previously or subsequently found to be active, failed to cause a sustained elevation in BP. Of the 7 unresponsive cat preparations, one was lethargic before being anesthetized; one was found to have had a hemorrhage from the ligature area of the previous nephrectomy; one was improperly pithed; two, following pithing, had undergone a somewhat prolonged period of very low BP (20-30 mm. Hg). In two animals nothing grossly abnormal was observable. Some of the cats with a very low BP following pithing were given 5-10  $\mu$ g. of epinephrine or 0.1 mgm. of p-hydroxy-a-methylphenethylamine hydrobromide (given as "Paredrine"). After the pressor effect had worn off the BP usually leveled off at 50-70 mm. Hg. In these animals the injection of active plasma gave sustained pressor responses which were in no way different from those observed in cats which had not exhibited very low initial blood pressures and were not given restorative injections. Among test preparations there occasionally were unaccountable variations in sensitivity to the pressor substance in that 4-5 ml. of active plasma was required in some cats to give the same sustained pressor response as that produced by 1-2 ml. of the same plasma when given to other test cats. There was no grossly apparent difference in the animal preparations to account for the observed variation in sensitivity.

The ability to respond with a sustained pressure elevation is not dependent upon the partial destruction of the central nervous system of the test cat. Five non-pithed anesthetized (pentobarbital sodium) "2- to 3-day nephrectomized" cats were injected with 2-10 ml. of active cat plasma and there resulted 30-70 mm. Hg increases in BP which were sustained for 1.25-4 hours (as long as the BP was recorded). The anesthetic did not appear to influence the response since each of 4 unanesthetized, nephrectomized cats, the femoral artery and vein of which were cannulated under procaine anesthesia, also exhibited sustained responses of comparable magnitude for as long as the BP was recorded (1-2.5 hrs.).

*Identity of the pressor principle.* Preliminary experiments indicate that its ability to cause a sustained elevation of BP in the nephrectomized cat preparation is not shared by angiotonin, epinephrine, pepsitensin, hydroxytyramine, or tyramine (see fig. 10). The conditions under which the pressor substance is found in the blood plasma (i.e., following a period of hypotension with the kidneys intact) suggest that renin could be the agent responsible for the sustained pressor



activity. Since the blood plasma of animals in the hypotensive state has been shown to contain renin (4, 5, 6), active dialyzed cat plasmas were assayed for their renin content.<sup>4</sup>

A small but measurable amount of renin was found in each of the active plasmas. A solution containing renin was then prepared from kidneys removed from normal cats, and standardized according to its ability to make angiotonin *in vitro*. Amounts of cat "renin" (kidney extract) and active plasma which possessed the same quantity of renin—as determined by their ability to make angiotonin—were injected intravenously into a pithed cat nephrectomized 48 hours before, and their pressor responses compared (fig. 6). The response to renin was characterized by a rise in BP with a rounded peak and a slow fall which was convex to the base line. The active plasma not only gave a much greater response but one which was sustained.

"Renin," as used by investigators for the study of its physiological effects, is in reality a mixture of the enzyme with unknown amounts of "impurities," the physiological actions of which are not known. The injection into "2-day nephrectomized" cats of an extract of cat kidneys which contains renin (as evidenced by its ability to form angiotonin *in vitro*) causes the typical curve shown in figures 6 and 10. However, the BP does not always return completely to the

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Fig. 7-10. Mean blood pressure tracings of pithed cats nephrectomized 2 days before (fig. 7, 8, 10) and one non-nephrectomized (fig. 9). Ordinate scales—mm. Hg. Time marker—1 minute.

Fig. 7. Comparison of blood pressure responses to i.v. injection of dialyzed plasmas obtained from (anesthetized) non-nephrectomized and nephrectomized cats subjected to prolonged hypotension by bleeding. 1, 2 ml. "control" plasma from non-nephrectomized cat, mean blood pressure, 140 mm. Hg; 2, 2 ml. plasma from same cat after bleeding to average blood pressure, 60 mm. Hg for 25 minutes; 3, 2 ml. plasma from terminal blood after 2 hours average blood pressure, 50 mm. Hg; 4, 2 ml. "control" plasma, same as 1; tracing paper changed and record interrupted for 1 minute at break; 5, 2 ml. "control" plasma from nephrectomized cat, mean blood pressure, 135 mm. Hg; 6, 2 ml. plasma from terminal blood of same cat after maintaining hypotension, average blood pressure, 40 mm. Hg for 5 hours; 7, 2 ml. terminal blood plasma from hypotensive, non-nephrectomized cat, same as 3 (32ZC-184).

Fig. 8. I.V. injection (1 and 2) of 2 ml. fresh plasma from terminal blood of cat (killed by DDT method) into pithed cat nephrectomized 2 days before (32ZC-127).

Fig. 9. I.V. injection into non-nephrectomized pithed cat of 2 ml. of same active plasma injected at 1 and 2, figure 8 (32ZC-128).

Fig. 10. I.V. injections into pithed cat nephrectomized two days before. 1, angiotonin; 2, pepsitensin; 3, tyramine hydrochloride; 4 and 6, renin extract from cat kidneys; 5 and 7, 2 ml. dialyzed plasma from terminal blood of cat killed by DDT method (32ZC-180).

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<sup>4</sup> Two sources of renin substrate were used:—one was dialyzed plasma obtained from cats nephrectomized 2 days before, and the other was a serum protein fraction prepared from hog blood by the method of Plentl and Page (7). For the renin assay, 3 cc. of the plasma or substrate solution plus 1 cc. of saline solution were incubated with 1 cc. of the active cat plasma for 15 minutes at 37°C. Dilute hydrochloric acid was added to pH 5.1-5.2 and the mixture placed in a boiling water bath for 10 minutes. The coagulated protein was removed by centrifuging and the clear supernatant fluid poured off and tested for angiotonin by intravenous injection into a pithed cat.

base level; a sustained elevation of 10–15 mm. Hg is sometimes observed. It is possible that renin may split another substrate and form, *in vivo*, an entirely different pressor substance which is much more stable than angiotonin. Equally tenable is the possibility that the “impurities” which are carried along with renin may contain substances which are either pressor in themselves or are capable of producing in the animal pressor substances which are relatively stable and capable of causing a very prolonged pressor action. Further experiments will be required for determining whether the principle herein reported is an enzyme or whether it is itself pressor.

*Chemical nature of the pressor principle.* Preliminary studies have demonstrated that the pressor material is destroyed at pH 5.0–7.0 when heated to 100°C. for 10 minutes; it is retained in a cellulose membrane when dialyzed against tap water for 3 days; it is precipitated between 0.3 and 0.6 saturation with ammonium sulfate; it is not completely precipitated by saturation with sodium chloride at pH 4.0. A considerable purification can be made by diluting the active plasma with an equal volume of 10 per cent NaCl solution and adding 5 ml. of glacial acetic acid to each 100 ml. of solution. After centrifugation the supernatant is dialyzed against tap water, centrifuged again, and then concentrated either by vacuum distillation or by precipitation with ammonium sulfate.

**DISCUSSION.** With no proof whatsoever that the sustained pressor principle herein discussed is identical with or even related to the hypothetical pressor agent responsible for hypertension, it is nevertheless of interest to examine the present experiments in terms of their possible relationship to the physiological mechanism of the initiation and possible abolition of experimental hypertension.

A pressor principle appears in the blood plasma of cats that have been subjected to a known period of hypotension as the result of bleeding, or that have gone through a period of hypotension of unknown duration while dying as the result of various procedures or from certain undiagnosed “natural causes.” The pressor agent has been found to be present in the plasmas from the blood of cats which have died with kidneys intact and not in the plasma from cats whose kidneys had been removed from 3 to 46 hours before death. The pressor substance was not found in the blood of normal living cats or in the blood of normal cats which had been killed “acutely.” These findings suggest that the pressor agent arises from the kidneys and is formed as the result of a low blood pressure and/or low blood flow within the kidneys, a concept which has also been advanced as the physiological mechanism for the elaboration of the “hypertensive agent.”

The substance found in the dying or dead cat's blood plasma, when injected into the blood stream of a “2-day nephrectomized” cat preparation causes a very marked and sustained elevation of blood pressure without appreciable change in heart rate. Injection of the same material in the same amounts into non-nephrectomized cats does not cause a sustained pressor response.

A speculative argument presented earlier in the paper supported the choice of the somewhat “chronic” (2-day) nephrectomized cat as the test preparation that would be most likely to respond to the “hypertensive” pressor agent by exhibiting a sustained pressure elevation if a sufficient amount of the substance

were injected. One of the bases for this hypothesis was the assumption that the kidneys, as suggested by many workers, are capable of antagonizing the "hypertensive" pressor agent, possibly by the elaboration of an inhibitor substance. To the extent that the pressor principle in cat plasma did not cause a sustained elevation of blood pressure in normal cats with kidneys intact, the experiments reported here suggest the possible existence of an analogous antagonistic mechanism.

#### SUMMARY

A pressor principle has been found in the terminal blood of cats which have died as the result of certain undiagnosed "natural causes," DDT poisoning, and prolonged hypotension resulting from the withdrawal of blood.

The pressor principle has not been demonstrated in the blood plasma of bilaterally nephrectomized cats which have died of DDT poisoning, prolonged hypotension or from uremia.

The pressor principle has not been found in the blood plasma obtained from normal living cats or normal cats which had been killed suddenly by various means. It is concluded that a moderately prolonged period of hypotension (with diminished blood flow and/or blood pressure within the kidneys) is necessary for the production of the pressor principle.

The pressor principle caused a sustained elevation of blood pressure in cats which were unanesthetized, anesthetized, or pithed, and had been bilaterally nephrectomized 2 days before. The injection of the same amount of plasma containing the pressor principle into non-nephrectomized cats did not cause a sustained elevation of blood pressure.

The pressor principle appears to be distinct from renin, angiotonin, pepsitensin, hydroxytyramine, or tyramine because of the difference in the contour of the pressor response, the duration of the pressor response, and the difference in the conditions under which the response is observed.

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# BREATH HOLDING AS A TEST OF PHYSICAL ENDURANCE<sup>1</sup>

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During World War I breath holding was used by the Royal Air Force of England as one of the tests of physical fitness (3). The subject was allowed to make a full exhalation and then, after a deep inhalation, he held his breath as long as possible. Passing time was set as 45 seconds. Hambley et al. (4) in 1925, in experimenting with this test, came to the conclusion that such a test was of no value for assessing physical fitness, because it only measured the ability to withstand the discomfort caused by breath holding. The use of the test in the R. A. F., however, continued until November, 1939. McCurdy and Larson (5) used a modified form of breath holding. The subject was required to blow through the small opening of a flarimeter, after a standard stepping-up exercise. They found a significant correlation between the time for swimming 440 yards by expert swimmers and breath holding time. They also observed that breath holding ability dropped after confinement in the infirmary and increased during training.

As a result of recent experiments Cureton (6) concluded that breath holding is a useful test of motor fitness when performed after running in place for 2 minutes. In April, 1945, at the meeting of the Joint Committee on Physical Fitness, sponsored by the National Committee of Physical Fitness of the Federal Security Agency and the American Medical Association, breath holding was again considered as one of the possible tests of physical fitness. The present study was motivated by that meeting and was undertaken in order to determine the value of various forms of breath holding as tests of physical fitness. Since previous investigators had considered breath holding as a test of cardio-respiratory fitness, it was decided to find the relationship between breath holding and 2 standard physical activities which place considerable demands upon the cardio-respiratory function. The activities selected for this purpose were a treadmill run and the Harvard step-up test.

**METHOD.** The following tests were used:

1. Breath holding in sitting position after 3 consecutive deep inhalations. Three trials were given.
2. Breath holding in sitting position after one full exhalation. Three trials were given.
3. Breath holding in sitting position after one inspiration immediately following one minute of prescribed exercise. The exercise consisted of stepping up and down on a 20 inch bench at the rate of 24 complete steps per minute—cadence 96 counts per minute. Stepping-up was done in the same manner as in the Harvard test (7).

<sup>1</sup> This study was made at the Army Air Forces School of Aviation Medicine, Randolph Field, Texas (1, 2).

4. Breath-holding while stepping up and down on a 10 inch bench at the rate of 24 complete steps per minute for as long as possible. Three deep inhalations were allowed immediately before the stepping-up exercise.

5. Same as No. 4, except that a 20 inch bench was used.

6. Harvard step-up test (7). The pulse rate was taken for 30 seconds, beginning 1 minute after exercise. The duration of exercise and the pulse rate were used in calculating the test score.

7. Running to exhaustion on a treadmill at a speed of 7 miles per hour and 8.6 per cent grade.

Forty-eight aviation students between the ages of 19 and 27 years were used as subjects. Each man was tested in a randomized order on 2 different days with an interval of 3 days between the tests. In order to avoid the effect of fatigue, the running and the Harvard step-up tests were not given on the same day. All testing was done in an air-conditioned laboratory at a temperature of 77°F. Testing time was recorded in seconds. Fractions of a second more than 0.5 were recorded as full seconds.

RESULTS AND COMMENTS. The coefficients of reliability of the various tests used in this study, as determined by the test-retest method, proved to be statistically significant.

1. Breath holding at rest after 3 inspirations,	$r = 0.89$
2. Breath holding at rest after 1 expiration,	$r = 0.56$
3. Breath holding after stepping-up on 20 inch bench,	$r = 0.78$
4. Breath holding while stepping-up on 10 inch bench,	$r = 0.66$
5. Harvard step-up test score,	$r = 0.73^2$

The reliability of breath holding while stepping up on 20 inch bench was not determined, but it is justifiable to assume that it would have been close to that obtained with the 10 inch bench, because both tests had the same statistical measures.

The reliability of the treadmill run was not tested in this study, but there is a sufficient amount of evidence in athletics and in scientific literature that running times are also fairly reproducible. For example, for a 300 yard run  $r = 0.86$ , 1000 yard run  $r = 0.80$  and for a mile run  $r = 0.89$  (9).

All data collected with each breath holding test were correlated with the treadmill running times; the Harvard step-up test scores; and the times obtained with breath holding at rest after 3 inspirations. The latter was done because breath holding after inspiration is the most common of the breath holding tests and for this reason it seemed logical to use it for comparison with the other respiratory tests. The pertinent statistical data are presented in table 1. As may be seen from this table, the coefficients of correlation between the breath holding tests and the treadmill running time and the Harvard test score are statistically not significant. For this reason breath holding tests cannot be used for prediction of endurance. The intercorrelations between the breath holding tests themselves are of interest. Only breath holding after stepping-up on a 20 inch bench showed a statistically significant correlation with both: the best

<sup>2</sup> Obtained on 187 men in a previous study (8).

and the average times of breath holding after an inspiration  $r = 0.77$  and  $r = 0.73$  respectively. The best time for breath holding after expiration and that after inspiration had an  $r = 0.35$ , which is of doubtful statistical significance, but the correlation of the average times gave an  $r = 0.61$ , which is statistically significant. The breath holding time while stepping-up on a 10 inch bench had an  $r = 0.55$  with the best breath holding time at rest after inspiration and only 0.32 with the average time of 3 tries. It is of interest that the breath holding times obtained while stepping-up on 10 and 20 inch benches were approximately the same, the means being respectively 44.2 seconds and 41.9 seconds, and the

TABLE 1  
*Statistical data*

TESTS	COEFFICIENTS OF CORRELATION WITH				THRESHOLD OF SIGNIFICANCE*	NO. OF SUBJECTS	TIME IN SECONDS		
	Treadmill run	Harvard score	Breath holding at rest, after inspiration				Range	Mean	Standard deviation
			Best time	Average time					
Breath Holding: 1 to 7									
1. After inspiration at rest, best time.....	0.21	0.23	0.89†		0.37	48	53-250	103.7	36.6
2. Average of 3 trials.....	0.30	0.24			0.37	48	43-208	89.4	30.5
3. After expiration, best time.....	0.06	0.09	0.35		0.37	47	15-74	43.0	13.6
4. Average of 3 trials.....	0.014	0.14		0.61†	0.37	47	14-66	34.2	10.2
5. After stepping up on 20" bench..	0.10	0.16	0.77†	0.73†	0.37	48	9-44	19.2	7.2
6. While stepping up on 10" bench..	0.23	0.09	0.55†	0.32	0.45	31	23-81	44.2	11.3
7. While stepping up on 20" bench..	0.12	0.21	0.27	0.25	0.45	31	27-103	41.9	13.8
Harvard Test: 8 and 9									
8. Pulse rate after test.....	0.40		0.17		0.39	46	104-156	129.4	13.8
9. Score.....	0.39	0.73	0.23	0.24	0.39	46	70-105	85.9	9.2
10. Treadmill Run.....		0.39	0.21	0.30	0.37	48	172-829	33.2	127.2

\* Value which coefficient of correlation should exceed to be significant at a 1 per cent level of probability.

† Statistically significant.

standard deviations being 11.3 seconds and 13.8 seconds. The test of the significance of the mean difference between these two tests showed that the difference was statistically not significant,  $t = 1.14$ , whereas  $t_{0.01} = 2.75$ . It was expected that the time for the 20 inch bench would be considerably shorter than for the 10 inch bench because the amount of mechanical work was doubled and, therefore, the intensity of chemical and reflex stimuli to the respiratory center was greater. This suggests a possibility that during the 20 inch bench test motivation becomes greater and the voluntary inhibitory effect upon the respiratory center is also increased. Some additional experimentation should be undertaken to probe this possibility.

## SUMMARY AND CONCLUSIONS

1. A group of 48 aviation students was used for this study.
2. The breath holding times obtained with five different tests were correlated with the maximum treadmill running times and the Harvard step-up test scores.
3. Since the coefficients of correlation thus obtained are statistically not significant, the breath holding ability cannot be used for prediction of either running endurance or Harvard step-up test score.

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## SOME CHARACTERISTICS OF GASTRIC SECRETION INDUCED BY MUSTARD OIL SUSPENSION<sup>1,2</sup>

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In a recent communication from this Laboratory (6) our observations on the consistency, opacity, color, and columnar cell content of gastric mucous secretion were reported. The material was obtained from Pavlov or Heidenhain pouch dogs in response to a number of topical stimuli. The list of agents included 1, gentle massage of the mucosa with a soft rubber catheter; 2, distilled water saturated with ether; 3, five per cent emulsion of clove oil in water; 4, fifty per cent ethyl alcohol; 5, isotonic NaCl (0.17 N); 6, hypertonic NaCl (0.5 N); 7, distilled water, and 8, no experimental stimulus (for control). With any one stimulus, some of the specimens were fluid whereas others were jelly-like; some were transparent or translucent, others were opaque; some contained few or no intact desquamated columnar cells as revealed by microscopic examination of mucous smears, whereas others contained many such cells. The response to the various stimuli differed only in respect to the percentage frequency with which each member of any pair of these characteristics occurred.

One of the numerous stimuli which have been employed by other workers for the investigation of mucous secretion in the digestive tract is 1 per cent aqueous mustard oil (allyl isothiocyanate) (1, 2, 3). This substance has generally been considered to be an acceptable agent for such studies. For this reason it was included in our original investigation, but the response to this irritant differed so radically from the others that it merited wholly separate consideration.

**PROCEDURE.** The experimental technique was essentially that employed for the other stimuli, and was previously reported in detail (6). The stimulating agent consisted of a 1 per cent suspension of mustard oil (allyl isothiocyanate) in water. In some of the experiments, this was prepared with the wetting agent Tergitol-Penetrant-4 to aid emulsification.<sup>3</sup> The volume of stimulus injected depended upon the capacity of the pouch; in no case was this less than 40 or more than 60 ml. Because of the great irritant action of mustard oil, the total dura-

<sup>1</sup> Preliminary reports of this work were presented before the American Society of Biological Chemists in 1941 (5) and the American Gastroenterological Association in 1944 (4).

<sup>2</sup> This investigation was supported in part by grants from the Anna Fuller Fund, Wyeth Inc., and the United Hospital Fund of N. Y.

<sup>3</sup> A concentration of  $\frac{1}{40}$  per cent Tergitol-4 was chosen, not only because it produced effective emulsification, but also because there was no mucus-stimulating action in control experiments using only this detergent at the above concentration. The secretion obtained after its administration resembled spontaneous secretion in secretory rate and appearance. However, control experiments with  $\frac{1}{4}$  per cent Tergitol-4 gave a rate of stimulation slightly greater than that for spontaneous secretion. Tergitol-Penetrant-4 was kindly supplied by its manufacturer, Carbide and Carbon Chemicals Corp., New York City.

tion of stimulation was usually only 5 minutes. In a few instances when it was 10 minutes, the total period was divided into two consecutive 5-minute intervals by the aspiration and reinjection of the material. This was done in order to refresh those portions which were in direct contact with the mucosa. Since the secretory response following a treatment continued for more than 5 hours, instead of 2-3 hours as with the other stimuli, the present experiments were stopped before the rate had fallen to its control (pre-stimulation) value. The duration of the experiments was not uniform, because of other objectives. The time interval for the collection of single samples also varied according to the requirements of the individual experiment. In all, 15 experiments were performed with mustard oil, using 7 Pavlov or Heidenhain pouch dogs. All of these animals had been employed for the experiments using the other agents. However, the mustard oil experiments were not performed in succession but were intermingled with the others.

**RESULTS.** *Duration and rate of secretion.* After application of the stimulus, about 10 minutes were required to set the dog up in the collecting stand and to drain most of the stimulus out of the pouch. During this combined period of stimulation and drainage (about 15 min.) some secretion undoubtedly took place. Hence it is impossible to determine a "latent period" between stimulation and beginning of secretion. Following drainage of the stimulating agent, secretion was collected for 5-15 minutes; this was designated the "initial specimen" of the experiment. The variations in secretory rate for subsequent collection intervals are illustrated in figure 1, which depicts the results of one of our best experiments. In general the duration of flow following a single application of the stimulating agent was 5 hours or more. The rate of secretion during the initial collection period was higher than during any subsequent one in all but 4 of the 15 experiments. Immediately following this initial high value, the rate began to fall off, the change being rapid at first and then progressively slower. The curvilinear trend depicted by the graph in figure 1 is fairly typical of the entire series of experiments. By replotting these data on semi-logarithmic paper (fig. 2) its exponential character becomes evident. In only 3 of the 15 experiments was the trend curve strikingly different—more or less flat—and in all of these cases the over-all rate of secretion was so low that they can be characterized as atypical.

For the 15 experiments included in the present series the highest initial rate of secretion was 17.4 ml. per 15 minutes; the mean of these 15 values was 11.1, with a standard deviation ( $S.D._M$ ) of 1.34 ml. A total of 107 specimens were collected in these experiments; the highest rate of secretion was 19.8 ml. per 15 minutes and the mean was 5.26 ( $S.D._M = 0.45$ ). All average values were calculated without weighting for volume or time-interval. An analysis of these several data, according to whether the mustard oil suspensions were prepared with or without Tergitol, revealed no significant difference which might be ascribed to the use of this wetting agent.

*Consistency of the secretion.* Specimens obtained in the experiments with mustard oil were generally fluid enough to drip continuously from the collecting catheter. Of 46 specimens collected with mustard oil in the absence of Tergitol,

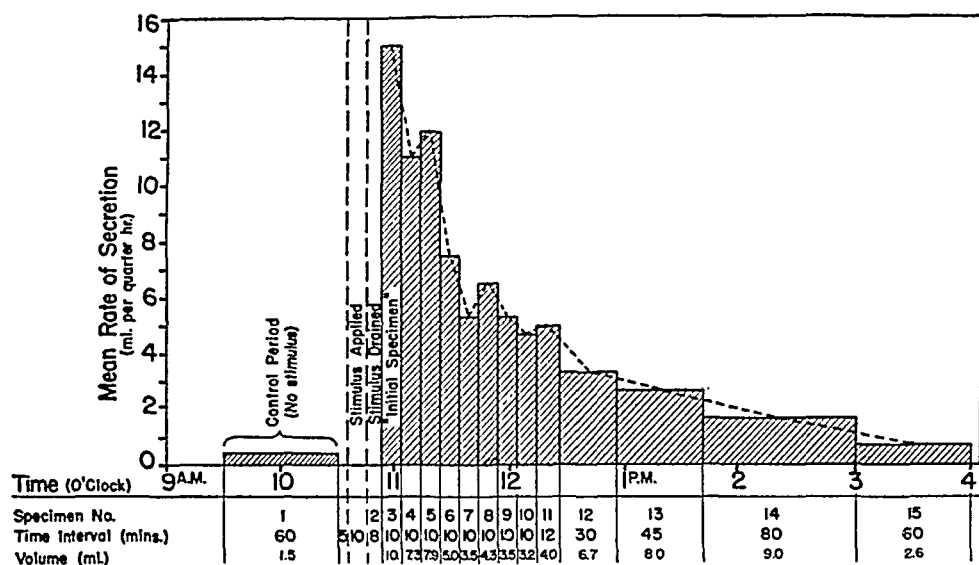


Fig. 1. Secretory response of dog's Heidenhain pouch to 1 per cent aqueous mustard oil suspension (expt. Mu.-278).

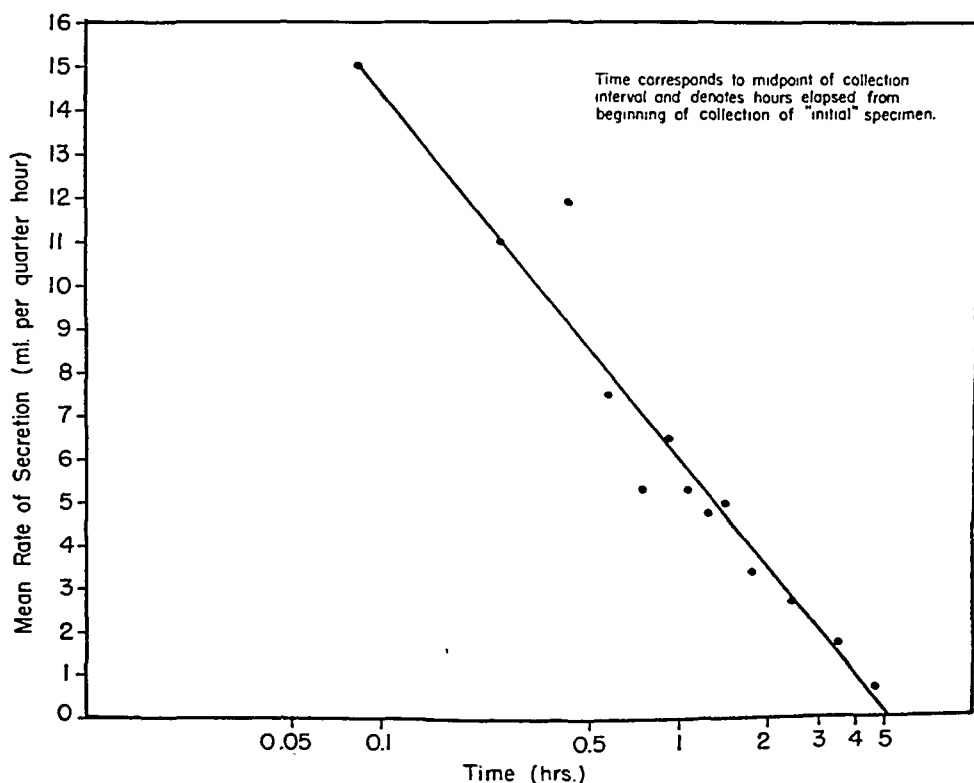


Fig. 2. Secretory rate data plotted against logarithm of time (expt. Mu.-278).

1 sample (2 per cent) was characterized as viscous or jelly-like. For the specimens obtained with the addition of Tergitol, the results were only slightly different; of a total of 61 specimens, only 6 (10 per cent) were viscous. The

difference between these two percentages is not significant statistically ( $P = 0.10$ ) but they are both considerably smaller than the control value of 45.7 per cent derived from spontaneous secretion.

In contrast with the fluid specimens obtained in response to the previous stimuli, those induced by mustard oil gelled on standing at room temperature. Sometimes this process started during the collection period; at other times it was observed 5 to 30 minutes thereafter. Usually the clot seemed to include the entire volume of specimen; on standing overnight in the refrigerator, however, it retracted and a supernatant layer of fluid was noted. In appearance and texture, this coagulum differed strikingly from jelly-like mucus in that it was translucent and much softer. Although it cohered on pouring, the mass was easily broken up and a uniform mixture created by vigorous shaking.

*Color.* The fluid obtained directly after stimulation with mustard oil had the amber appearance of blood plasma. Some time before the third hour following removal of the stimulus, the color became faint pink; in subsequent specimens, this continued to deepen to a decided red. The sanguinous origin of this color was confirmed both chemically (by guaiac test) and microscopically (by the presence of erythrocytes). Tergitol exercised no appreciable influence on this sequence of colors.

*Opacity.* Although experiments with and without Tergitol manifested no difference in regard to bleeding, they did with regard to opacity of the specimens. In the absence of this emulsifier, the specimens were almost always transparent or translucent; only 2 in a group of 39 (5 per cent) could be described as opaque. When Tergitol was used, however, the proportion rose to 23 in 61 (38 per cent) a difference which is clearly significant. In both groups of experiments, the typically clear specimens sometimes contained small opaque particles in suspension. However, without Tergitol the frequency of occurrence of such particles, and their density per specimen, were characteristically low—frequently near zero—whereas when mustard oil-Tergitol was used, both frequency and density were high. Control experiments with Tergitol alone in a concentration of  $\frac{1}{40}$  per cent gave rise to secretion resembling, both in rate and in appearance, that obtained spontaneously.

*Presence of columnar cells.* Systematic examination of mucous smears was performed in 6 of the 7 experiments without Tergitol, and yielded 31 specimens for which both opacity and cell content were recorded. All but one of these were non-opaque; all of them were found to contain very few or no columnar cells on examination of representative smears. However when smears were deliberately prepared to include one or two of the opaque particles referred to in the previous paragraph, many cells were noted. Microscopic examination of these particles after staining revealed them to be bits of surface epithelium, such as were obtained with some of the other stimuli and described in the previous report. Those particles were surrounded by many individual columnar cells—particularly dense at the periphery of the particle, less so at a slight distance.

*DISCUSSION.* Mustard oil (allyl isothiocyanate) in aqueous suspension has been used by several investigators in studies on mucous secretion in the digestive tracts of experimental animals. From such reports, however, it is apparent that

this agent induces an inflammatory reaction as well as a secretory response of the mucous epithelium. In our own experience, the material obtained from pouches stimulated with mustard oil differs markedly from that secreted after application of other agents employed as mucus stimuli. This can be demonstrated by a comparison of the data obtained in the present investigation with those previously reported from this Laboratory (6) for spontaneous secretion, mechanical stimulation, and contact with aqueous solutions and suspensions having various irritant potencies.

Concerning rate of secretion, none of the 425 specimens obtained with the stimuli used previously ever yielded values higher than 2.3 ml. per quarter hour, and the majority of them were well below 1.0, whereas the highest value for mustard oil secretion was 19.8 ml. and the mean was 5.3. Furthermore, the entire duration of the former experiments—i.e., the time required for the secretory rate to return to its control value, the rate of spontaneous secretion—rarely exceeded three hours. In contrast with this, the mustard oil experiments lasted at least twice as long, and even after this extended interval, the rate had not yet fallen to the control level. No comparison of the two sets of experiments is possible in regard to the shape of the secretory-time curves, because of the quantitative inadequacy of the rate data of the experiments with the other stimuli. As previously explained, this arose from the gelatinous character of a majority of these specimens. Hence, judging by time and volume relations alone, it might be inferred that the mustard oil suspension is a far more potent stimulus than any which we have previously investigated. Other characteristics of the response discussed in the following paragraphs, however, suggest a radically different conclusion.

With only rare exception, the consistency of the specimens of secretion collected in the mustard oil experiments was recorded as fluid. Regardless of the presence or absence of Tergitol, only 6.5 per cent were viscous or jelly-like as compared with 45.7 per cent previously reported for the specimens of "spontaneous secretion". In fact, the former value is lower even than that obtained after application of *isotonic saline* solution (13.3 per cent), but the difference between the two has no statistical significance. Our earlier work led to the conclusion that the incidence of mucus of high viscosity is probably correlated directly with the irritant power of the stimulus. In contrast with this, we find that a 1 per cent suspension of allyl isothiocyanate—which may be more irritating than most or even all of these other stimuli—yields only a negligible incidence of jelly-like specimens, and therefore might be classified as a mucus stimulus of very slight potency.

In regard to opacity, it was found that the mustard oil suspension prepared with Tergitol gives a higher incidence of opaque specimens than does the Tergitol-free suspension—38 per cent as compared with 5 per cent. The influence of the detergent will be discussed later. However, even the former value is considerably less than the values obtained with 50 per cent ethyl alcohol, hypertonic NaCl solution, water saturated with ether, and 5 per cent clove oil suspension in water—which vary between 82 and 96 per cent. Furthermore, the over-all

incidence of opaque specimens in all mustard oil experiments (with and without Tergitol) is almost identical with that for spontaneously secreted mucus—25 per cent and 27 per cent respectively.

Unfortunately, only one of the experiments with Tergitol-mustard oil included microscopic examination for columnar cell content. In the 6 experiments without Tergitol, however, the incidence of specimens with "many" columnar cells was only about 3 per cent, and the correlation of high (and low) cell content with opacity (and non-opacity) was extremely high. This percentage value is of the same order of magnitude as that obtained with isotonic saline (7 per cent), and considerably less than that for spontaneous secretion (30 per cent). However, small isolated areas of high columnar cell density were sometimes found, even in slides with an otherwise low density, but this localized occurrence of individual cells probably results from an occasional single clump of epithelial cells rather than from a diffuse, generalized desquamation. It appears, therefore, that the mustard oil suspension without Tergitol does not induce the extensive desquamation characteristic of other stimuli, and though the addition of Tergitol does cause desquamation to some degree, it is not nearly as extensive as with the other stimuli. It is not unlikely that the clumps of surface epithelium which occur occasionally in the mustard oil specimens are shed "spontaneously", since such clumps are seen with about the same frequency as in the specimens of "spontaneous" secretion. From this it seems evident that the mustard oil induces little or no loosening of the intercellular cement substance.

Concerning the influence of Tergitol-4 on the action of the allyl isothiocyanate, it is clear that there is none so far as the time relation of fluid output, the extent of bleeding, or even the consistency of the secretion are concerned. However, in regard to the opacity a difference exists between the experiments with and without the wetting agent, specimens from the former being slightly, but significantly, more opaque than those from the latter. Although the data on the microscopic appearance of the Tergitol-mustard oil specimens is deficient, there is evidence to indicate that they contain a slightly greater number of clumps of columnar cells than do the others. A similar correlation between opacity and incidence of epithelial cells was demonstrated in our report with the other stimuli as well. It is difficult to see why the presence of Tergitol should result in this slight increase in cell content of the specimens, particularly since control experiments with the detergent alone yielded material which differed in no way from that previously described as "spontaneous secretion". It may be that the detergent loosens portions of mucus adherent to the mucosa in the resting state, together with occasional bits of epithelium previously desquamated and enmeshed in this mucus. On the other hand, the peculiar response to the mixture of mustard oil and Tergitol may result from the potentiation of one by the other, either by actual chemical combination or because of the surface activity of the wetting agent.

Thus, in terms of consistency, opacity, and columnar cell content, a 1 per cent emulsion of mustard oil may be deemed to be no more potent as a mucus stimulus than is isotonic saline. On the other hand, both rate and duration of secretion

suggest an intensity of stimulation by the mustard oil emulsion which exceeds by far that manifested by any other agent which we have so far studied. This is in accordance with the fact that 1 per cent mustard oil would ordinarily be classified by pharmacological criteria as considerably more irritating than any of these other mucus stimuli. It may be thought that the striking difference between the results with mustard oil and those with the other agents arose from a pathological condition of the mucosa, e.g., a gastritis, which obtained at the time the present experiments were performed but was absent during the experiments previously reported. This is precluded, however, by the fact that the mustard oil experiments were irregularly distributed among all the others in point of time of their performance.

In attempting to resolve this paradox, several factors must be kept in mind. The total volume of secretion obtained in response to a single application of the mustard oil suspension is many times greater than that following application of even the most potent of the stimuli previously employed. Also in both groups of experiments, metachromatically-staining material (presumably mucin) was repeatedly found to be present, but the quantity in the mustard oil specimens appeared to be considerably less than in the others. Unfortunately, at the time these investigations were performed, there was available to us no acceptable analytical method for determining concentration of mucin, so that our comparisons have been made on a qualitative rather than a quantitative basis. In spite of the presence of mucin, however, the mustard oil specimens bear a closer resemblance to blood plasma or transudate than to fluid mucus. This is evidenced by their color—which is amber during the first hours of collection, and pink to red during the latter part—and also by their slow congealing shortly after collection. This gradual yet persistent appearance of blood is another manifestation of an inflammatory reaction induced by the stimulus. There is little likelihood that the bleeding results from traumatization by the collecting apparatus. Such oozing of blood was never observed with the other stimuli, and constitutes further evidence that transudation (or exudation) bears the major responsibility for the lack of concordance between the mustard oil experiments and the others.<sup>4</sup> Thus the paradox can be resolved by the recognition of the fact that we are dealing here with a mixture of transudate (or exudate) and mucous secretion, in which the former is in considerable excess over the latter.

#### CONCLUSIONS

It may be concluded from the foregoing that a 1 per cent emulsion of mustard oil in water is a very poor stimulus for gastric mucus secretion, in comparison with the agents previously studied. On the contrary, this agent is an active mucosal irritant, giving rise to large amounts of a "secretion" which consists for the most part of serous transudate (or exudate). The persistently low

<sup>4</sup> It is noteworthy that the pH-values of mustard oil specimens are generally greater than 7.5, the uppermost limit reported by Menkin (7) for lymph and inflammatory exudate. Many of the values fall between 8.0 and 8.5. The significance of these findings in relation to the present study will be considered in a later report.

viscosity, low order of opacity, low incidence of columnar cells, and probable small mucin content—all indicate that mustard oil in this concentration fails to evoke the usual responses of a true mucus stimulus. On the other hand, the high volume, rate, and duration of secretion; the resemblance in color of early specimens to blood plasma; the almost invariable appearance of fresh blood, increasing in intensity with progress of the experiment—all these, together with the observations on viscosity, opacity, etc. support the view that the "secretion" is a transudate or exudate. Undoubtedly, some mucus is present in the "secretion", but the proportion seems to be very small indeed. It is evident, therefore, that mustard oil emulsion should not be used as a stimulus for investigation of mucous secretion in the stomach.

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# THE RÔLE OF THE MESENTERIC CIRCULATION IN THE IRREVERSIBILITY OF HEMORRHAGIC SHOCK<sup>1</sup>

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Evidence recently accumulated by several investigators in this laboratory has re-emphasized the concept that sequestration or "pooling" of blood in certain vascular channels may be a significant factor in the irreversible shock which characterizes the standardized hemorrhagic procedure here employed for dogs. By "irreversible" is meant the unfavorable response to transfusion which follows a prescribed period of hypotension, namely, a mean arterial blood pressure of 50 mm. Hg for 90 minutes and 30 mm. Hg for 45 minutes (1). Alexander (2) from a study of arterial pulse contours has found indirect evidence that although general compensatory mechanisms may be adequate after transfusion certain discrete vascular beds appear to be dilated, a situation which would favor pooling in the dilated areas. Eckstein and co-workers (3) found that inferior vena cava flow declined rapidly in the post-infusion period to about half of the control flow, suggesting the possibility of pooling in vascular areas supplying the inferior vena cava. This interpretation of their work was supported by the finding of Foreman (4) that redistribution of blood occurred in the course of hemorrhagic shock, and at autopsy much more blood than normal was recovered from the regions supplying the inferior vena cava.

That the mesenteric vascular bed may be a significant area of pooling was indicated by the finding of Wiggers, Opdyke, and Johnson (5) that portal pressure was significantly elevated following re-infusion of blood, signifying venous congestion of the mesenteric bed. The concept of mesenteric vascular pooling is further supported by the appearance of the upper small intestine of the dog at autopsy following a typical course of hemorrhagic shock (1). The mucosa is typically congested and edematous, with characteristic hemorrhagic areas, varying in intensity. In severe cases free blood is found in the lumen.

The purpose of the present study has been *a*, to determine whether the hemodynamic characteristic of the mesenteric circulation during the course of hemorrhagic shock in dogs permits the conclusion that pooling of blood may occur here, and *b*, to study the possible mechanism by which such pooling may occur.

**EXPERIMENTAL PROCEDURE.** The following surgical procedures were necessitated in dogs anesthetized with approximately 280 mgm. of sodium barbital per kgm. of body weight administered intravenously. For purposes of measuring mesenteric blood flow and portal pressure a ventral abdominal incision was made to permit splenectomy and approach to the portal vein. After splenectomy the main splenic vein was prepared for cannulation. The excised spleen was drained

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into heparin solution and the recovered blood was filtered and subsequently reinfused into the animal before the experiment was begun. The method for measurement of mesenteric blood flow required placement of a ligature under the portal vein near its point of entry into the liver. This was done without undue exposure of the intestine by reflecting backward the upper intestine with a retractor padded with saline-moistened gauze. Placement of the ligature with the aid of an aneurysm needle was usually accomplished in a brief time interval. The ligature was then passed to the surface through a brass sleeve and the intestinal retractor was removed. It was concluded that intestinal manipulation was minimal during preparation of the animal and did not contribute unduly

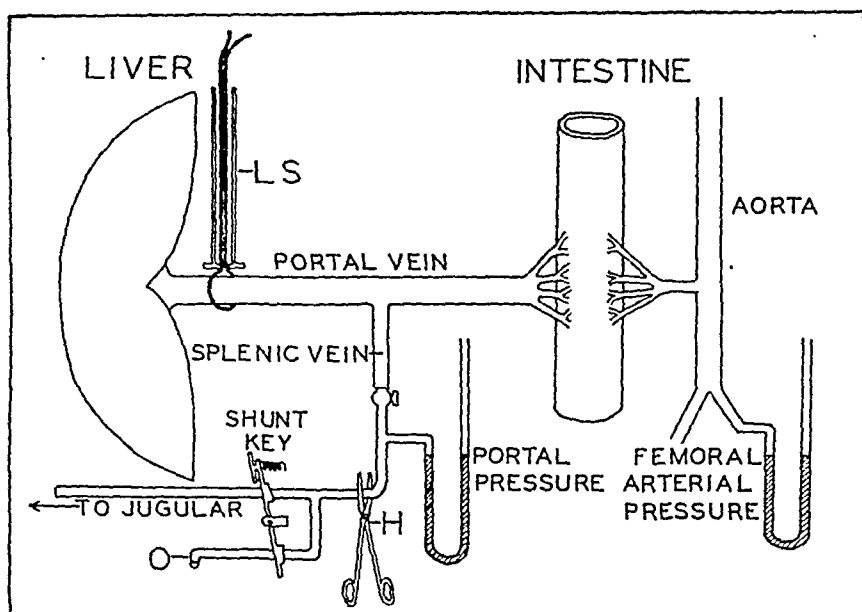


Fig. 1. Schematic diagram of the arrangement for measuring mesenteric blood flow. To make a flow determination the liver shunt (LS) is closed, and flow is directed for a half minute into the jugular circuit. Then timed samples are diverted by depressing the shunt key. The outflow orifice, O, is set at the level of the vena cava which serves as the zero pressure reference point for the manometers. Between flow readings and when portal pressure is measured a hemostat, H, keeps the jugular circuit closed.

to the shock state. Much more intestinal manipulation seems to be required to induce shock experimentally: Average time intervals of approximately  $3\frac{1}{2}$ ,  $5\frac{1}{2}$ , and 7 hours were used by different groups of investigators (6, 7, 8). Finally, an external jugular vein was cannulated for reinfusion of blood and both femoral arteries were cannulated, one for bleeding, and the other for registration of arterial blood pressure.

*Method for measuring mesenteric blood flow.* The following technique was employed for measurement of mesenteric blood flow (see fig. 1): a tubular brass cannula, 6 cm. long and 3-4 mm. in diameter, was passed down the splenic vein so that its opening lay near the junction with the portal vein. The other end of the cannula was connected to an external circuit emptying into the jugular vein.

This circuit was kept closed except when flow was measured. Before proceeding to measurement of flow the animal was heparinized with a priming dose of 4 mgm. per kgm. of body weight (additional amounts of 5 mgm. total dosage were given every half hour thereafter). To make a blood flow determination the jugular circuit was opened and the ligature under the portal vein was pulled up against the brass sleeve, closing the portal system and thus shunting blood through the jugular circuit. The brass sleeve was rigidly supported in the proper position so that the portal vein ligature could be adjusted without traction or frictional irritation of the intestine. After a time interval of 30 seconds to permit stabilization of flow through the external jugular circuit depression of a shunt key for a brief interval diverted blood flowing through the jugular circuit into a graduate cylinder. By accurately timing the period of outflow mean mesenteric blood flow in cubic centimeters per minute could be calculated. The portal loop was then released, the jugular circuit was closed, and the drawn blood was immediately reinfused via the jugular vein. The outflow cannula was continuously set at the level of the vena cava, which was taken as the zero pressure reference point for the manometers. It must be emphasized that the liver received its usual supply of blood via the portal system except during the brief periods when blood flow determinations were made. Individual readings and periods of observation were suitably spaced so that no consequential interference with hepatic circulation resulted.

The advantage of this method is that mesenteric blood flow is measured at the rate determined by the resistance characteristic of the mesenteric vascular bed alone, and is independent of the influence of possible changes in hepatic vascular resistance while the flow determination is being made. With the technique described mesenteric flow is in effect emptying into the vena cava (via the jugular vein) during periods of observation. The term "mesenteric blood flow" is used in preference to "portal blood flow" because the latter implies that the volume of blood normally emptying into the portal vein passes through the liver before emptying into the inferior vena cava. The mesenteric veins from the intestines are the important contributors to this flow but some blood comes from the stomach and pancreas.

Since mesenteric venous outflow empties against resistance offered by the liver during normal circulation it is obvious that flow measured in the above manner can be expected to exceed that encountered in normal portal flow. During the control periods of 21 experiments of this report the average flow was 19.0 cc. per minute per kgm. of body weight (S.D.  $\pm 6.3$ ) with the mesenteric outflow at the level of the vena cava. In another series of 12 experiments in which mesenteric venous outflow emptied against an increased resistance averaging 11.0 mm. Hg (approximating normal portal pressure), produced by elevation of the outflow cannula, the average flow was reduced to 8.7 cc. per minute per kgm. (S.D.  $\pm 3.4$ ). The average mean arterial pressure was approximately the same in these two groups (131 and 134 mm. Hg respectively). It is evident that moderate changes in hepatic resistance and portal pressure could appreciably influence mesenteric flow, a conclusion also reached by Wiggers and co-workers (5). Since the object

of the present investigation has been to study changes in flow as influenced solely by mesenteric resistance, it has been found expedient to adopt the procedure described above.

Mean arterial blood pressure was registered continuously from the femoral artery with a damped mercury manometer. For convenience, a mercury manometer was also used for registration of portal pressure. With careful use of the mercury manometer the trend in portal pressure during hemorrhagic shock has been found to be the same as that recorded optically by Wiggers and co-workers (5), but averaged 1 or 2 mm. Hg higher, since they took their measurements during the expiratory pause of respiration.

From the above measurements two other hemodynamic variables were calculated, viz., the total mesenteric resistance and the ratio of portal pressure to mean arterial pressure. Total mesenteric resistance was calculated in the customary manner from the ratio of *mean arterial blood pressure* (MABP) to *total mesenteric flow in cubic centimeters per minute*:  $\frac{\text{MABP}}{\text{flow/cc./min.}}$  and is expressed

in peripheral resistance units (PRU), where one PRU =  $\frac{1 \text{ mm. Hg}}{1 \text{ cc./min.}}$ . The ratio of portal pressure to mean arterial blood pressure, PP/MABP, is considered significant because elevation of this ratio should favor pooling by acting to dam blood in the mesenteric vessels when arterial inflow is adequate.

A modification of the standardized hemorrhagic procedure (1) was that arterial pressure during the hypotensive phases was kept at approximately 60 and 40 mm. Hg, instead of the customary 50 and 30 mm. Hg. This was deemed necessary because of the surgical procedures and splenectomy.

**RESULTS.** A. *The hemodynamics of the mesenteric circulation during hemorrhagic shock.* Classification of fifteen animals on the basis of survival time shows that four died during the 40 mm. Hg period, four expired within an hour after reinfusion, and seven lived longer than one hour after reinfusion. Six of the latter group developed typical hemorrhagic shock, with an average post-infusion survival time of 2 hours and 40 minutes. One animal of prolonged survival was sacrificed with no conclusive evidence of shock.

Figure 2 shows a representative experiment of typical hemorrhagic shock in which circulatory failure developed gradually following reinfusion. Upon bleeding to the 60 mm. Hg arterial pressure level mesenteric blood flow was immediately reduced from the control average of 26.0 to 7.7 cc./min./kgm. During the first hour flow gradually increased to 9.0 cc./min./kgm., while arterial pressure was maintained at the 60 mm. level by small additional bleeding increments. Upon further bleeding to the 40 mm. level flow decreased to 7.8 cc./min./kgm. and continued to decrease throughout this period, being only 3.7 cc./min. just before reinfusion. It is noteworthy that the greatest reduction in flow occurred typically during the period of drastic hypotension, averaging in this animal 23 per cent of the control during the last half hour of the 40 mm. period.

Upon reinfusion of blood arterial pressure rose rapidly to 110 mm. Hg, then

declined briefly to about 100 mm. Hg where it remained for eight minutes (see phase A-B of fig. 2). During this period mesenteric flow was rapidly restored to a peak of 27 cc./min./kgm., but coincidental with a second phase of increasing arterial pressure to 123 mm. Hg mesenteric flow decreased rapidly to 19.0 cc./min./kgm. Then followed a more gradual decline to 11.2 cc./min. at 4½ hours post-infusion when mean arterial pressure was 75 mm. Hg.

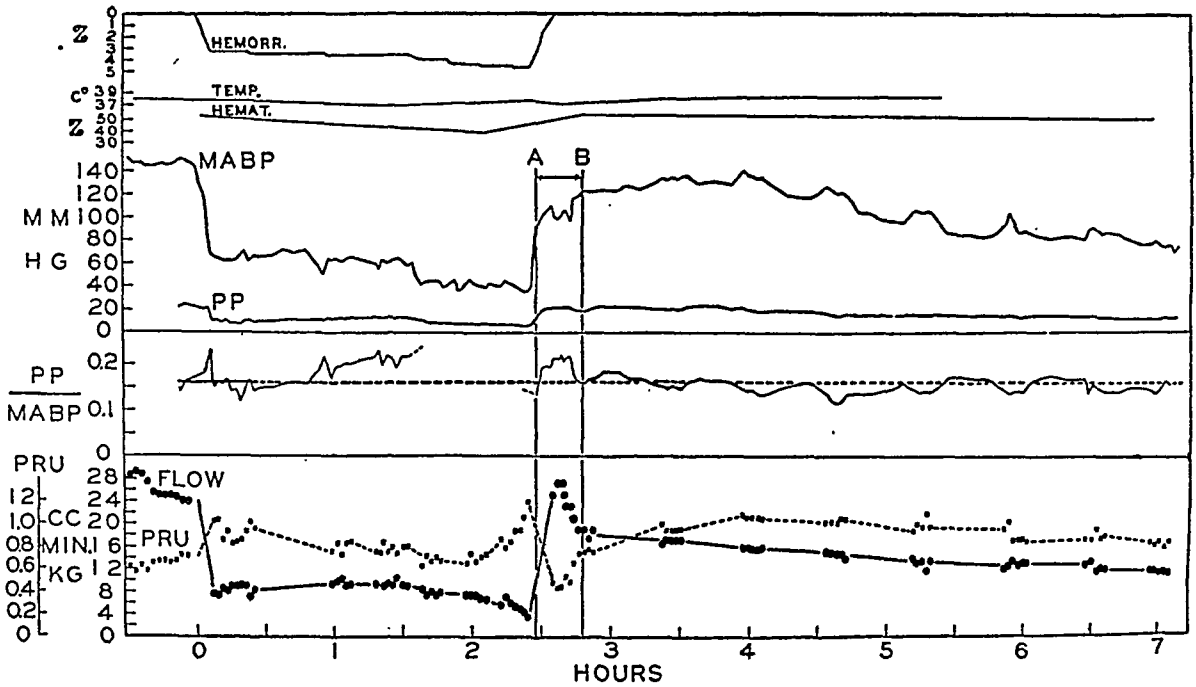


Fig. 2. A representative experiment illustrative of typical hemorrhagic shock. *MABP*: mean arterial blood pressure; *PP*: portal pressure; *PRU*: peripheral resistance units, a measure of total mesenteric vascular resistance. The solid line in the plot of the ratio  $PP/MABP$  represents the average value for the control and observations taken immediately after hemorrhage early in the 60 mm. period. It is extended to the right as a dashed line to serve as reference for further experimental changes. The phase A-B represents a critical phase of mesenteric pooling (see text). Amount of hemorrhage is expressed as per cent of body weight, and the point where hemorrhage was begun is taken as zero time. Control periods averaged 10 minutes in all experiments (time scale exaggerated 2.5× in figure). Weight, 8 kgm. At autopsy, intestinal mucosa showed 2 plus congestion.

Mesenteric vascular resistance exhibited an immediate increase from the control average of 0.654 to 1.00 PRU following the initial hemorrhage, then gradually decreased throughout the 60 mm. and early 40 mm. periods, but continued to remain above the control average. Late in the 40 mm. period resistance again increased to a peak of 1.2 PRU just before reinfusion. This trend of mesenteric resistance during hypotension exemplified well that observed in other typical shock experiments. After reinfusion it fell rapidly below the control average where it remained for 8 minutes (see phase A-B), then increased parallel with the second phase of increased arterial pressure. Mesenteric resistance then remained

well above the control value throughout the remainder of the experiment although it showed a later tendency to decrease somewhat, a trend occurring in varying degrees in other experiments.

The ratio PP/MABP showed only moderate changes in this animal. It characteristically increased during the 60 mm. Hg period and remained elevated throughout hypotension, a trend noted in varying degrees in all animals. Because portal pressure increased relatively more than arterial pressure following reinfusion the ratio remained elevated above control for a brief period (phase A-B) but was soon restored to the control average (dashed line in the fig.) for the remainder of the experiment.

It is considered that phase A-B is symptomatic of a critical period of mesenteric vascular pooling because *a*, the ratio PP/MABP is elevated; *b*, mesenteric resistance is low; and *c*, the rate of arterial inflow is high. All shock experiments classed as typical agreed in showing a phase of decreased mesenteric resistance immediately after transfusion, and at this time the ratio PP/MABP was elevated in all.

In figure 3, part A, an experiment is plotted which resembles closely the above in hemodynamic trends during the hypotensive period, but in which the animal died precipitantly  $1\frac{1}{2}$  hours after reinfusion following an initial adequate recovery of arterial pressure. As in the above experiment, a critical phase (A-B) was seen immediately after transfusion, marked by an initial rapid decline of arterial pressure from 109 to 93 mm. Hg, followed by a second phase of increase to 111 mm. Hg twelve minutes later. Early in phase A-B mesenteric flow recovered to 16.5 cc./min./kgm., 87 per cent of the control, and remained elevated near this value despite later decline in arterial pressure.

Mesenteric vascular resistance typically decreased following transfusion during phase A-B to a value of 0.423 PRU as compared to the control average of 0.627, then showed a gradual return to the control resistance value. Contrary to the trend seen in the experiment of figure 2 mesenteric resistance did not exceed the original control value during the post-infusion period, but rather entered a second phase of decrease (C-D in fig. 3, A) terminating in a value of only 0.200 PRU. It appeared that phase C-D was a second critical period of mesenteric pooling which developed during precipitant circulatory failure and that this was largely responsible for the fact that arterial pressure declined rapidly to a fatal termination.

Another difference from the experiment of figure 2 was that the ratio PP/MABP was elevated above the control value throughout the post-infusion period and significantly so during the critical periods A-B and C-D. If the inference is correct that mesenteric pooling occurs during these periods then a possible answer for the more limited survival of this animal is offered, for the factors of elevated portal-arterial pressure ratio and reduced mesenteric resistance operate to a greater degree than in the animal of figure 2.

The relation of elevated PP/MABP ratio and decreased mesenteric resistance to circulatory collapse is even more evident in animals which died in fulminant shock. A representative experiment of this type appears in figure 3, part B.

A striking feature of this experiment was that mesenteric resistance never increased but continually fell throughout the course and was lowest during the post-infusion period, averaging half of the control resistance. Blood flow was therefore comparatively high during the hypotensive period and recovered to virtually the control rate following transfusion, despite recovery of arterial pressure to only 61 mm. Hg. The ratio PP/MABP was more than twice the control average during the post-infusion period, and since mesenteric resistance was continuously reduced the entire post-infusion period apparently represented a critical phase of mesenteric pooling. Since the portal/arterial pressure ratio began to increase during the 60 mm. period and mesenteric resistance decreased simultaneously it is probable that pooling may also have occurred during the

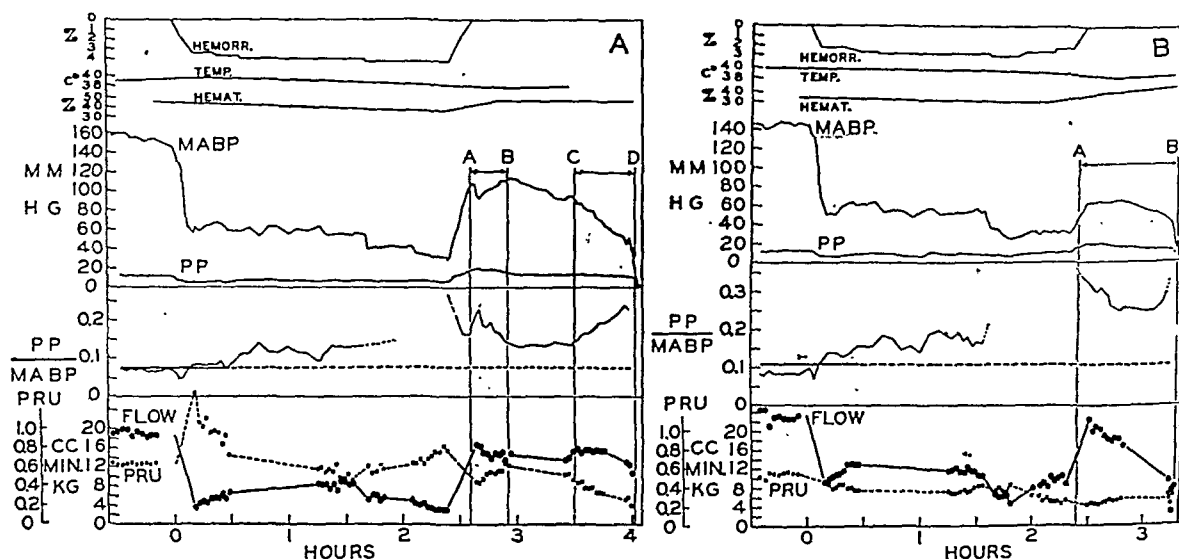


Fig. 3. Representative experiments showing different trends of hemorrhagic shock. *Part A:* This figure illustrates one of the shorter experiments classified as typical shock. Phases A-B and C-D are considered symptomatic of mesenteric pooling (see text). Autopsy findings showed 3 plus congestion of the intestine. Weight: 12.5 kgm. *Part B:* This experiment illustrates fulminant shock. At autopsy intestinal congestion was of grade 4 plus. Animal weight: 11.2 kgm.

hypotensive stages of this experiment. Such observations characterized the course of three animals that died during the 40 mm. period. Other animals of short survival exhibited mixed trends between this type and the more typical pattern of shock as described in the first two experiments.

*B. The effect of experimental elevation of portal pressure on mesenteric blood flow, mesenteric vascular resistance, and arterial blood pressure.* An allied series of six experiments is included in the present report in which animals were not subjected to hemorrhage, but in which the effect of experimentally elevated portal pressure on mesenteric blood flow and resistance, with subsequent influence on arterial pressure was examined. This was done to test the hypothesis that increase in hepatic vascular resistance during the course of hemorrhagic shock might be the primary factor responsible for portal pressure elevation and subsequent congestion of the mesenteric vessels by a back pressure effect.

Experimentally, this was accomplished by proper adjustment of the loop under the portal vein so that portal pressure was maintained at about 20 mm. Hg, simulating the condition which was found to exist in the post-infusion period of the animals subjected to hemorrhagic shock. Control arterial pressure averaged 137 mm. Hg and control portal pressure averaged 11.5 mm. Hg in this group. Experimental elevation of portal pressure to 20 mm. Hg thus increased the ratio PP/MABP to 0.146, a value compatible with the average found in the post-infusion period of typical hemorrhagic shock.

The sequence of events can best be described by reference to figure 4 which shows two representative experiments. Part A of the figure shows an experiment in which the animal terminally exhibited signs of circulatory collapse, an event which occurred in two other animals of the group. Part B of the figure shows an experiment in which the effects were less marked, and in which the animal showed good recovery, true in the remaining two animals.

Of particular interest in these experiments was the parallel downward trend of mesenteric resistance and arterial pressure following elevation of portal pressure. This is interpreted to mean that the decrease in mesenteric resistance promoted by portal pressure elevation must be accompanied by pooling of blood in this area, loss of which from the active circulation results in decline of arterial pressure. (Note how mesenteric flow was maintained despite decline in arterial pressure because of dilatation or increase in total calibre of mesenteric vessels.)

In all cases vasomotor compensation was initiated as evidenced by a phase of increasing mesenteric resistance beginning at an average time of about one hour after portal pressure elevation and paralleling this a rise in arterial pressure. This phase was not permanent in some animals for, as stated, in three circulatory collapse and death followed in from 1 hour, 40 minutes, to 5 hours, 20 minutes. At autopsy the intestinal mucosa showed congestion entirely comparable to that seen following a course of hemorrhagic shock of grade 3 plus or 4 plus in these three animals. The other three animals (exemplified by fig. 4, part B) did not show as great a decline in arterial pressure following portal pressure elevation, showed better compensatory mechanisms and had good arterial pressures when sacrificed. In these intestinal changes were minimal, about 1 plus. The reason for the difference in response of these animals is not apparent.

DISCUSSION. In confirmation of the work of Wiggers, Opdyke and Johnson (5), a dominant and consistent finding in all animals which died in shock was an increase in portal pressure relative to mean arterial pressure, or more specifically, an increase in the ratio PP/MABP. This ratio continued to rise throughout the hypotensive period of standardized hemorrhage and was significantly elevated above control in the post-infusion period. In fact, the degree of increase may be a determining factor in the survival time for it appeared to be highest in animals of short survival, and was not elevated in an animal in which shock was questionable. Mesenteric resistance in the latter animal remained continuously above control until sacrificed  $4\frac{1}{2}$  hours post-infusion when arterial pressure was 101 mm. Hg.

Increase in the ratio PP/MABP could result from *a*, increase in hepatic resistance, or *b*, decrease in mesenteric resistance. It is likely that both are con-

cerned, but the view is favored from the present experiments that increased hepatic resistance is primary because increase in the ratio may not be accompanied by simultaneous decrease in mesenteric resistance, which is true during the hypotensive stages of typical shock. But when arterial inflow is adequate, as occurs when blood is transfused in animals subjected to hemorrhagic hypotension, or when portal pressure is artificially elevated in animals not subjected to hemorrhage, it leads secondarily to decreased mesenteric resistance. The nature of this decreased mesenteric vascular resistance can only be conjectured with the

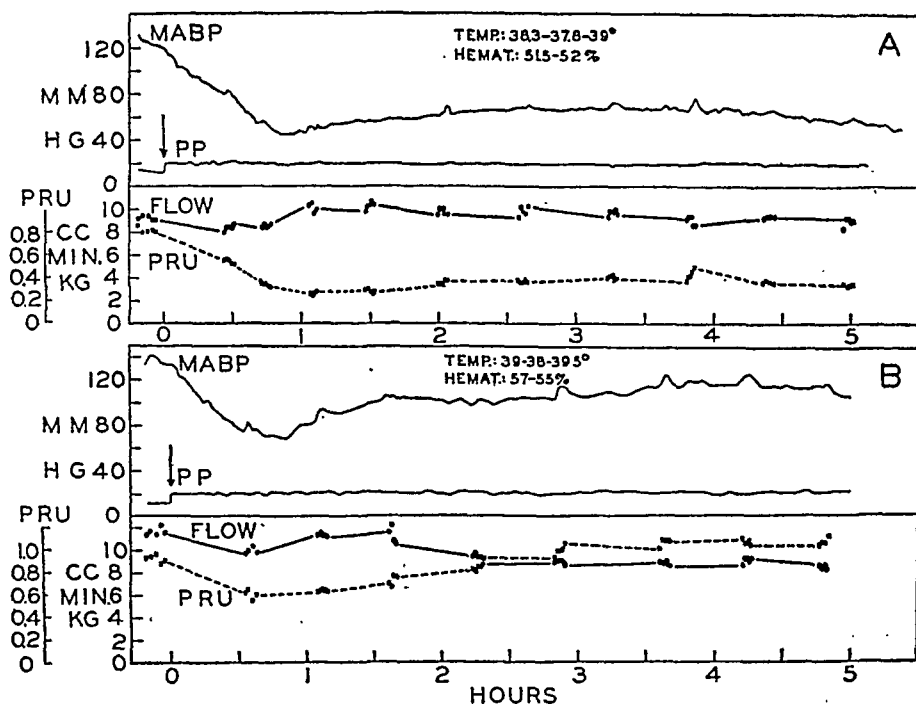


Fig. 4. These representative experiments demonstrate the effect of experimental elevation of portal pressure on mesenteric blood flow, mesenteric resistance, and mean arterial blood pressure in animals not subjected to hemorrhage. Portal pressure is elevated to 20 mm. Hg at the arrow. In animal A (16.2 kgm.), there were terminal signs of circulatory collapse, and at autopsy the intestine showed 3 plus congestion. Animal B (12.4 kgm.) showed good vasomotor compensation. At autopsy, intestine showed minimal (1 plus) change.

present lack of knowledge of the relationship of mesenteric blood flow to arterial perfusion pressure, but it seems safe to assume that it results from increased total calibre of the mesenteric vascular bed, either by dilatation of individual vessels, or by opening of additional vascular channels or shunts. The site of such dilatation cannot be definitely ascertained, but because of their low elasticity coefficient the collecting venules and capillaries are the most likely regions for stagnation and pooling, visual demonstration of which during shock appears to have been supplied by Zweifach and his co-workers (9, 10).

It was noted that mesenteric resistance was increased throughout the hypoten-

sive period in the group classified as typical in our series. Such initial increase in mesenteric resistance, taken to be largely the result of primary arteriolar vasoconstriction, although it limits pooling during the hypotensive phase, must certainly promote anoxia in these tissues, and may thus establish conditions which later favor pooling (e.g., atony of the mesenteric vessels). During reinfusion of blood, mesenteric resistance declined markedly from the value during hypotension, and this is believed to be the result of greatly increased arterial inflow into atonic mesenteric vessels against increased hepatic resistance. Rapid sequestration of blood must have occurred at this time for vasomotion appeared to be intensified as the result of blood loss by pooling, since increase in mesenteric resistance soon followed. But because no permanent benefit was derived from this event the indication was that blood remained trapped in mesenteric capillaries and venules coincidentally. On the basis of Chambers and Zweifach's analysis of the topography and function of the mesenteric capillary circulation (11), this may be explained by conjecturing that by extension of arteriolar constriction to main A-V capillaries or shutting down of larger arterio-venous shunts, blood is diverted and trapped in the true capillaries.

Two fundamental aspects of the problem require further discussion: the cause of the elevated portal pressure and increased PP/MABP ratio, and the mechanism of decreased mesenteric resistance. Wiggers, Opdyke, and Johnson (5) from their study of portal pressure gradients concluded that hepatic vascular resistance is increased during the course of hemorrhagic shock, and thus becomes an initiating factor in portal pressure elevation. Increase in hepatic resistance in response to hemorrhage is not surprising for other organs respond similarly, viz., the kidney (12, 13), the hindlimb (3, 14), and the spleen (15).

Two leading explanations are feasible to explain the decrease in mesenteric resistance: *a*, that vasodilator or "vasodepressor" substances are formed during the course of shock (possibly in the liver) which may influence the mesenteric vascular bed (9, 16), and *b*, that elevated portal pressure resulting from increased hepatic resistance tends to reduce mesenteric resistance by a mechanical back pressure effect, a conclusion favored here and supported by the experiments in which portal pressure was experimentally elevated. The specific mechanism of this effect is not known, but is probably a manifestation of the plastic property of the smooth muscle of the blood vessels. The vulnerability of the mesenteric vessels to this influence was strikingly demonstrated by the fact that elevation of portal pressure from a control average of 11.5 mm. Hg to 20 mm. Hg was enough to begin a chain of events which eventuated in some animals in circulatory collapse. The general trend of hemodynamic changes which follows experimental elevation of portal pressure resembles those following reinfusion of blood during the hemorrhagic shock procedure (except that events are more condensed in the latter); strongly suggesting that basically similar factors are operative.

The concept of intravascular sequestration or pooling of blood as the cause of irreversible shock is of course not a new one (see review by Wiggers, 17). Particularly striking in this connection are the experiments of Janeway and Jackson (18) and Erlanger and Gasser (19), in which shock was produced by partial

occlusion of the inferior vena cava. The latter workers convincingly demonstrated the presence of intense congestion of the upper intestinal mucosa as the result of the back pressure effect, and related the phenomenon to generalized sequestration of blood, concluding that reduction of effective blood volume by this means was a significant factor in circulatory collapse. The experiments of Elman and Cole (20) and Boyce, Lampert, and McFetridge (21) with complete portal obstruction in dogs have more specifically involved the mesenteric vascular bed in such obstructive pooling. The dogs of Elman and Cole survived for an average time of 66 minutes, and those of Boyce et al. for 87 minutes after complete occlusion of the portal vein. By comparing the weight of the entire gastro-intestinal tract after this shock procedure with the weight of controls they concluded that a blood volume of 5.2 and 3.05 per cent of body weight in their respective studies was trapped in the vessels of the gastro-intestinal tract, enough to account for death by intravascular hemorrhage. These workers did not, however, study the circulatory dynamics of their procedure beyond recording abrupt and precipitant collapse of arterial blood pressure. It is likely that complete stoppage of portal blood flow must have created severe liver anoxia, a factor complicating the interpretation of their results.

Our experiments of partial obstruction of the portal vein so as to elevate the portal/arterial pressure ratio to a value about 50 per cent above control have created a state more comparable to that found after reinfusion of blood in animals subjected to hemorrhagic hypotension, establishing a more physiological basis for comparison. The similarity of hemodynamic changes in these experiments to those found in the post-infusion phase of hemorrhagic shock is evident, and analysis of hemodynamic characteristics of mesenteric circulation during hemorrhagic shock has made possible a better understanding of the mechanism involved. It is conjectured that reduction of effective blood volume by mesenteric pooling puts further serious strain on the animal already subjected to a period of stagnant anoxia with its damaging effect on many organ systems, and such blood loss undoubtedly contributes greatly to final respiratory, vasomotor, or cardiac failure.

#### SUMMARY

The behavior of the mesenteric circulation suggests that mechanisms are operative which favor sequestration or pooling of blood in mesenteric vessels, contributing to the irreversibility which characterizes the standardized hemorrhagic shock procedure. In animals which die fulminantly, mesenteric vascular resistance declines continually during the hypotensive period which, combined with an elevated portal/arterial pressure ratio, favors mesenteric pooling as a feature of final circulatory collapse.

In more typical cases of better survival mesenteric resistance increases during the hypotensive period, but a brief decline in the average below control follows reinfusion of blood. This is believed to represent a critical period of mesenteric pooling, for arterial inflow is rapid against increased portal resistance. Then a phase of increased mesenteric resistance soon develops, coincident with which

arterial pressure is well maintained for a time. This latter phase of increased resistance probably results from enhanced vasomotion stimulated by decrease in effective circulating blood volume by pooling in mesenteric vessels, but does not correct for the deficit.

Elevation of the portal/arterial pressure ratio is thought to play an important rôle in mesenteric pooling by a back pressure effect, and that increase in hepatic vascular resistance is the initiating factor. To test this hypothesis, in a group of animals not subjected to hemorrhage, portal pressure was experimentally elevated to a degree comparable to that seen in the post-infusion period of animals subjected to the hemorrhagic shock procedure. This resulted in a decrease in mesenteric resistance thought to favor mesenteric pooling, for it was accompanied by a variable decline of arterial pressure in all. Some of the animals went into circulatory collapse, and at autopsy showed the congested intestinal mucosa which characterized the findings in hemorrhagic shock.

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